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VOL. XLI—No. 2

Issued August 1, 1916

BALTIMORE, U. S. A.

1916

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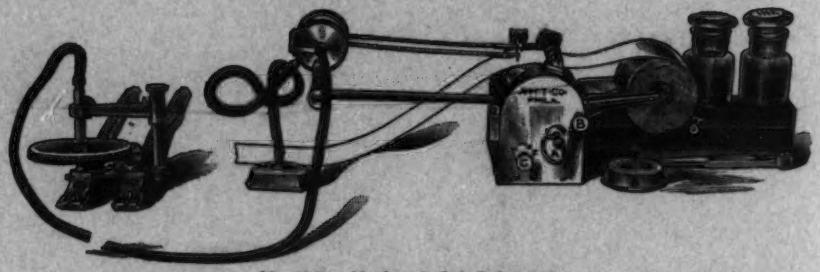

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THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 41 AUGUST 1, 1916 No. 2

SOME METABOLIC EFFECTS OF BATHING IN THE GREAT SALT LAKE. II

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Received for publication, May 29, 1916

In a previous paper (1) it was shown that bathing in the strong brine of Great Salt Lake increased the urinary elimination of nitrogen 5 to 7 per cent, and that of sodium chloride 21 to 27 per cent in two subjects on a uniform diet. To determine whether these findings are significant and constant, and whether they are causally related, a second experiment was carried out along similar lines on the same subjects. The uniform diet was as follows:

	MORNING		NOON AND EVENING	
	Subject 1	Subject 2	Subject 1	Subject 2
Toasted wheat biscuit	30 grams	30 grams		
Strawberries	50 grams	50 grams		
Graham crackers	90 grams	60 grams	120 grams	90 grams
Butter	25 grams	20 grams	25 grams	25 grams
Peanut butter	20 grams	10 grams	20 grams	10 grams
Sugar	5 grams	5 grams		
Milk	465 cc.	465 cc.	465 cc.	465 cc.

Protein per day (calculated), Subject 1, 102 grams; Subject 2, 85 grams.
Calories per day (calculated), Subject 1, 3455; Subject 2, 2857.

The experiment was divided into three periods; (1) a fore period of five days, (2) a bathing period of four days, and (3) a final period of three days. The urine was collected in 24-hour samples on six of the days and in three to four hour samples on the other six days. Of these latter samples 24-hour composites were also made. Analyses

were made for total nitrogen (Kjeldahl), ammonia (Folin), creatinine (Folin), uric acid (Benedict-Hitchcock) and chlorides (Dehn-Clark). All the determinations were in duplicate except those of total nitrogen and chlorides on the short period samples.

The variation in the urine constituents is shown graphically in figure 1, and the analytical data from which the figure was made, with record of temperature and length of baths, are given in table 1. In making the averages for the fore period the data of the first two days were discarded since they were influenced by the preceding mixed diet and by small changes in the uniform diet which were found necessary for the satisfaction of the subjects. The data of the period samples, taken on days 5 to 7 and 9 to 11 are given in tables 2 and 3, and are shown graphically in figure 2. Of these six days, 6, 7 and 9 are bathing days. In the figure the time of the bath and its duration are indicated by the line *B*.[1]

Referring first to the data in figure 1 and table 1 it appears that the average daily total nitrogen excretion increases slightly in the bathing period over the fore period, which is in agreement with previous findings. There is a gradually decreasing nitrogen output during the fore period and a gradually increasing nitrogen output in the bathing period. The maximum is reached on the day following the last bath, in both subjects, and in the final period the average daily nitrogen excretion exceeds that of either of the preceding periods, i.e., the high level reached at the end of the bathing period is practically maintained in the final period, a result differing from previous findings.

The other nitrogenous urinary constituents show so little variation as to permit of no conclusions. The very slight rise in creatinine excretion noted in the bathing period of the former experiment is not observed here.

In contrast to the results of the former experiment in which the average excretion of sodium chloride was increased during the bathing period there is shown in the present data a decreased output of sodium chloride during the bathing period, as compared with the fore period, while in the final period the average output is above that of the fore period.

The excretion of nitrogen and of chlorides in the case of both subjects shows a striking uniformity throughout, a progressively decreas-

[1] It is perhaps unfortunate that absolute uniformity was not observed in the duration of these periods but the value of the date is not materially diminished on this account.

TABLE 1

Subject 1

DAY OF EXPERIMENT	DATE	TOTAL NITROGEN	AMMONIA	URIC ACID	CREATININE	SODIUM CHLORIDE	TEMPERATURE OF		LENGTH OF BATH
							Air	Water	
		grams	*grams*	*grams*	*grams*	*grams*			*min.*
1	June 3	13.79	0.47	0.55	1.24	8.75			
2	June 4	13.23	0.37	0.55	1.30	9.02			
3	June 5	12.58	0.33	0.65	1.24	9.82			
4	June 6	12.15	0.34	0.58	1.35	9.09			
5	June 7	11.90	0.32		1.40	6.87			
Average 3–5...		12.21	0.33		1.33	8.59			
6	June 8	12.47	0.29	0.55	1.32	6.59	25	24.5	25
7	June 9	12.56	0.31	0.39	1.29	7.50	24	24	40
8	June 10	12.72	0.30	0.42	1.29	8.40	23	24	45
9	June 11	13.56	0.32	0.41	1.31	8.68	24	26	45
Average.....		12.83	0.31	0.44	1.30	7.79			
10	June 12	13.70	0.32	0.41	1.27	8.83			
11	June 13	13.32	0.34	0.42	1.29	8.84			
12	June 14	13.49	0.31	0.45	1.31	8.64			
Average.....		13.50	0.32	0.43	1.29	8.77			
Subject 2									
1	June 3	9.44	0.32	0.52	0.92	8.54			
2	June 4	9.93	0.29	0.56	0.91	8.16			
3	June 5	9.18	0.24	0.38	0.92	8.02			
4	June 6	9.53	0.30	0.36	0.99	7.40			
5	June 7	8.55	0.33		0.96	5.10			
Average 3–5...		9.09	0.30		0.94	6.84			
6	June 8	8.85	0.27	0.29	0.93	4.20	25	24.5	25
7	June 9	8.86	0.27	0.39	0.93	5.16	24	24	30
8	June 10	9.49	0.19	0.38	1.02	7.43	23	24	45
9	June 11	10.84	0.24	0.48	1.02	8.75	24	26	35
Average.....		9.51	0.24	0.39	0.98	6.39			
10	June 12	11.16	0.25	0.35	0.98	8.32			
11	June 13	10.91	0.28	0.39	0.99	8.79			
12	June 14	10.65	0.25	0.37	1.03	8.94			
Average.....		10.91	0.26	0.37	1.00	8.68			

TABLE 2

Subject 1

DAY OF EXPERIMENT	PERIOD	TOTAL NITROGEN		SODIUM CHLORIDE		CREATININE	
		Per period	Per hour	Per period	Per hour	Per period	Per hour
5 (no bath)	8.00–11.00	1.48	0.494	1.19	0.395	0.20	0.068
	11.00– 3.00	2.12	0.530	1.83	0.456	0.25	0.062
	3.00– 6.00	1.29	0.429	0.70	0.234	0.17	0.057
	6.00–10.00	2.46	0.614	1.26	0.314	0.24	0.060
	10.00– 8.00	4.30	0.430	1.95	0.195	0.49	0.049
	For 24 hours	11.64		6.90		1.36	
	Composite	11.90		6.87		1.41	
6 (bath)	8.00–11.00	1.51	0.504	0.83	0.278	0.18	0.059
	11.00– 4.00	2.81	0.562	1.72	0.345	0.29	0.059
	4.00– 7.00	1.96	0.653	1.47	0.491	0.18	0.059
	7.00–10.00	1.62	0.540	0.86	0.288	0.17	0.055
	10.00– 8.00	4.59	0.459	1.74	0.174	0.51	0.051
	For 24 hours	12.49		6.64		1.32	
	Composite	12.47		6.59		1.32	
7 (bath)	8.00–12.00	1.68	0.421	1.07	0.267	0.21	0.053
	12.00– 3.30	2.00	0.571	1.37	0.392	0.21	0.059
	3.30– 6.30	2.13	0.709	1.98	0.661	0.18	0.060
	6.30– 9.30	1.76	0.586	0.90	0.298	0.17	0.057
	9.30– 8.00	5.00	0.476	2.07	0.198	0.50	0.048
	For 24 hours	12.57		7.39		1.27	
	Composite	12.56		7.50		1.29	
9 (bath)	8.00–12.35	2.31	0.505	1.75	0.380	0.27	0.060
	12.35– 4.20	2.34	0.625	1.62	0.431	0.22	0.059
	4.20– 7.35	2.48	0.762	2.58	0.793	0.20	0.061
	7.35–11.15	2.14	0.584	1.21	0.331	0.19	0.053
	11.15– 8.00	4.37	0.499	1.55	0.177	0.43	0.050
	For 24 hours	13.64		8.71		1.32	
	Composite	13.52		8.68		1.31	
10 (no bath)	8.00–11.40	2.02	0.552	1.53	0.418	0.21	0.058
	11.40– 3.40	2.58	0.644	2.18	0.544	0.24	0.060
	3.40– 6.45	2.00	0.649	1.29	0.419	0.18	0.057
	6.45–11.30	3.10	0.653	2.25	0.474	0.27	0.057
	11.30– 8.00	4.04	0.476	1.55	0.183	0.38	0.045
	For 24 hours	13.75		8.80		1.28	
	Composite	13.70		8.83		1.27	
11 (no bath)	8.00– 1.30	3.24	0.589	3.01	0.547	0.35	0.064
	1.30– 4.30	1.91	0.638	1.48	0.492	0.17	0.058
	4.30– 7.15	1.57	0.571	1.16	0.420	0.15	0.055
	7.15–11.15	2.59	0.646	1.52	0.380	0.23	0.058
	11.15– 8.00	4.03	0.461	1.68	0.192	0.41	0.047
	For 24 hours	13.34		8.84		1.31	
	Composite	13.31		8.84		1.29	

TABLE 3

Subject 2

DAY OF EXPERIMENT	PERIOD	TOTAL NITROGEN		SODIUM CHLORIDE		CREATININE	
		Per period	Per hour	Per period	Per hour	Per period	Per hour
5 (no bath)	8.00–11.00	1.14	0.381	1.12	0.371	0.14	0.048
	11.00– 3.00	1.50	0.376	1.42	0.356	0.17	0.043
	3.00– 6.00	0.80	0.267	0.58	0.192	0.12	0.040
	6.00–10.00			0.95	0.238	0.17	0.043
	10.00– 8.00	3.39	0.339	1.12	0.112	0.36	0.036
	For 24 hours			5.18		0.97	
	Composite	8.55		5.10		0.96	
6 (bath)	8.00–12.00	1.41	0.352	0.82	0.205	0.17	0.042
	12.00– 4.00	2.09	0.523	0.91	0.227	0.18	0.044
	4.00– 7.00	1.28	0.427	0.77	0.257	0.13	0.042
	7.00–10.00	1.12	0.372	0.49	0.163	0.11	0.038
	10.00– 8.00	3.35	0.335	1.23	0.123	0.38	0.038
	For 24 hours	9.24		4.22		0.97	
	Composite	8.85		4.20		0.93	
7 (bath)	8.00–12.00	1.18	0.296	0.86	0.214	0.16	0.040
	12.00– 3.30	1.44	0.411	1.05	0.301	0.15	0.043
	3.30– 6.30	1.48	0.494	1.13	0.376	0.14	0.045
	6.30– 9.30	1.28	0.426	0.61	0.203	0.11	0.038
	9.30– 8.00	3.64	0.347	1.50	0.143	0.38	0.036
	For 24 hours	9.02		5.15		0.94	
	Composite	8.86		5.16		0.93	
9 (bath)	8.00–12.40	2.08	0.445	2.27	0.486	0.23	0.049
	12.40– 4.20	1.79	0.488	1.49	0.407	0.16	0.043
	4.20– 7.35	1.77	0.545	2.17	0.670	0.14	0.044
	7.35–11.15	1.56	0.426	0.93	0.254	0.14	0.038
	11.15– 8.00	3.62	0.414	1.94	0.202	0.34	0.039
	For 24 hours	10.82		8.80		0.99	
	Composite	10.85		8.75		1.02	
10 (no bath)	8.00–12.00	1.72	0.430	1.79	0.447	0.18	0.044
	12.00– 3.00	1.45	0.484	1.42	0.474	0.12	0.040
	3.00– 6.30	1.65	0.470	1.00	0.314	0.14	0.040
	6.30–11.30	2.54	0.508	1.54	0.307	0.20	0.040
	11.30– 8.00	3.76	0.443	2.37	0.279	0.33	0.039
	For 24 hours	11.12		8.22		0.96	
	Composite	11.16		8.32		0.98	
11 (no bath)	8.00– 1.30	2.51	0.457	2.81	0.512	0.25	0.045
	1.30– 4.30	1.52	0.507	1.64	0.546	0.14	0.045
	4.30– 7.15	1.28	0.464	0.88	0.321	0.11	0.041
	7.15–11.15	2.06	0.515	1.20	0.299	0.16	0.041
	11.15– 8.00	3.67	0.420	2.32	0.265	0.36	0.041
	For 24 hours	11.04		8.85		1.02	
	Composite	10.91		8.79		0.99	

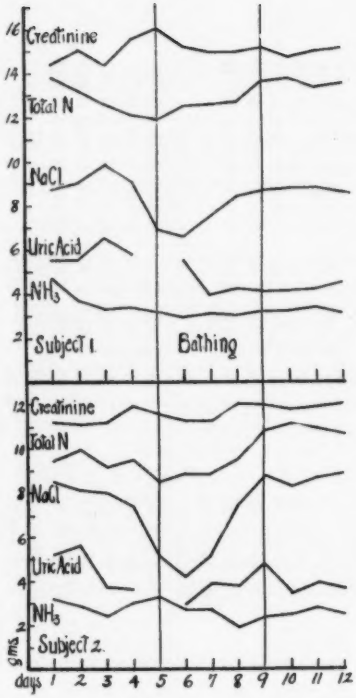

Fig. 1. Total nitrogen and sodium chloride are in grams, ammonia, uric acid and creatinine in tenths of grams, and all to the same base line except creatinine for which the zero is raised two units.

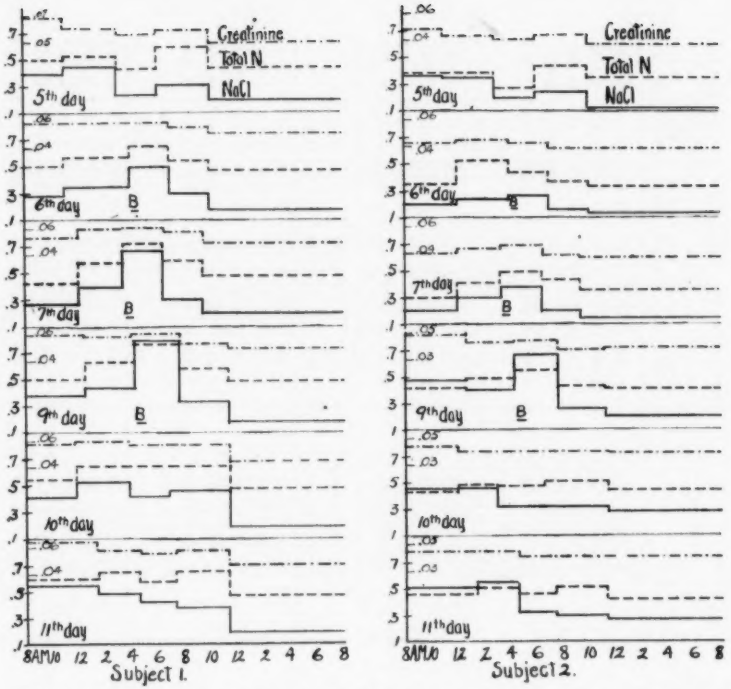

Fig. 2. Total nitrogen and sodium chloride in tenths of grams, creatinine in hundredths of grams.

ing output during the fore period, a progressively increasing output during the bathing period, which level is maintained during the final period.

The course of the chloride curves could be accounted for as follows: the uniform diet evidently contained less salt than the ordinary mixed diet which preceded it, so that during the fore period the effect of a lessened chloride intake gradually appears as a decreased salt excretion. With the beginning of bathing this relative retention of salt gradually gives way to increasing salt excretions which reach their maximum on the last day of bathing and remain at this level, or even slightly exceed it in Subject 2, in the final period. Nitrogen elimination follows practically the same course and this parallelism in nitrogen and chloride excretions was an unusual and unexplained finding in the previous experiment.

An examination of the data from the short period samples brings this out more clearly, and offers further evidence on the immediate effects of bathing. In figure 2 the third period on days 5, 10 and 11 (no bath) shows a marked difference from the same period on days 6, 7 and 9 (bath). When no bath was taken the rate of chloride and nitrogen excretion was lower during this period than during the preceding one, and usually lower than the rate in the succeeding period. On bathing days the rate of chloride and nitrogen excretion during this third period (at the beginning of which a bath was taken) was, in all cases but one, considerably higher than the rates observed both in the preceding and in the succeeding period. The progressively increasing daily output of nitrogen and chloride during the bathing period, as shown in figure 1, is largely caused, according to figure 2, by an increase in the rate of excretion immediately following the bath. The effect of the bath on metabolism is therefore primarily immediate though not entirely so; in both subjects the rate of excretion of nitrogen during the night period rises steadily reaching its maximum after the last bath, or on the day following, and the same statement may be made with reference to the chloride excretion of Subject 2.

An absorption of salt by the skin during the bath might explain the course of the chloride excretions as an attempt to restore chloride equilibrium, but the mechanism by which nitrogen excretion could be similarly involved is not clear. If the bathing brings about a mobilization of chlorides or their redistribution between the tissues and body fluids so as to cause their excretion, it would appear as if such a process were accompanied by an increased protein degradation or a

flushing out of already existing decomposition products of protein. If on the other hand the metabolism of nitrogen is primarily affected by the bathing the increased chloride excretion is far above the amount that would be associated with a decomposition of body protein to the extent necessary to account for the higher urinary nitrogen. It is, in fact, entirely probable that body protein remains untouched by the response of the organism to the bath, at least as far as glycogen formation is concerned. In three experiments on the influence of cold (8° to 12°C.) baths on the glycogen content of man Lusk (2) found glycogen formation from protein in one case, an individual under normal weight. In those experiments the intestine was free from carbohydrates and while no values for body temperature are given, the general and intense shivering noted in his subjects never obtained in these brine baths and the alimentary tract was never free from carbohydrate at the time of the bath. This of course does not exclude the possibility of other influences outside of g ycogen formation, which might modify protein metabolism. Whether these parallel findings on chloride and nitrogen excretion as modified by bathing depend upon the bath as such or upon the salt water must be determined by further experiments.

A chance deviation from the regular routine of the subjects is responsible for the suggestion that the increased nitrogen and chloride excretion by way of the kidneys might be merely the evidence of a lessened skin excretion. On the fifth day, which was warm, exercise in the form of tennis for an hour and a half was taken during the third collecting period, 3 to 6 p.m. Inasmuch as the exercise in the water on bathing days was not otherwise compensated for it was felt that this exercise, which was not violent, could be indulged in with impunity, but this was evidently not the case with reference to the skin excretion judging from the urinary nitrogen and chloride for the period. The hourly rate in this period was lower for this day than for the corresponding period on any of the non-bathing days in which period samples were taken.

Information upon the nitrogen and chloride content of the perspiration is somewhat fragmentary because of the variety of conditions under which observations have been made, but is sufficient to warrant the general statement that sweat usually contains from 0.05 per cent to 0.2 per cent of nitrogen and 0.5 per cent sodium chloride as a maximum concentration at the height of secretion rate (3, 4). Since Argutinsky (5) found 0.7 grams of nitrogen in the perspiration

collected during a seven-hour walk on a hot day there would have been about 0.15 gram nitrogen and 0.38 gram sodium chloride[2] in the perspiration excreted during an hour and a half of tennis, values which will explain the diminished amounts of urinary nitrogen and sodium chloride in the third period of the fifth day. That the cooling of the skin by bathing should prevent the elimination of perspiration sufficient to account for the higher urinary concentration in nitrogen and chlorides in the third period of the bathing days is scarcely a justifiable conclusion, and this question must be subjected to further investigation. The fact that body temperature is not markedly affected by the bath (1) and that the increasing chloride and nitrogen excretion during the bathing period continues at approximately its highest level during the final period indicates that other factors besides the urine-sweat partition are operating.

The rate of creatinine excretion as shown by the data obtained from the short period samples does not indicate that the bathing had any influence upon it; the data rather emphasize the constancy of the excretion rate of creatinine.

Several blood pressure measurements before, during and after the bath were made by means of a Tycos sphygmomanometer and auscultation over the radial artery. The values (systolic) obtained for Subject 1 were uniformly 110, for Subject 2, 105, and the expected rise in blood pressure during the bath was not shown by this somewhat approximate method.

SUMMARY

Two subjects were maintained on a uniform diet for twelve days. A bathing period of four days followed a fore period of five days, with a three day final period. The urine was analyzed in 24-hour periods on all of the days and in short periods (3 to 4 hours) on three bathing and on three non-bathing days. In agreement with former results the progress of the bathing period was attended by increased nitrogen and salt excretion, which, in this case, however, persisted through the final period. The three-hour period during and immediately following

[2] This calculation for sodium chloride is made on the assumption that the nitrogen excretion of 0.7 gram was 0.2 per cent of the total perspiration, which would therefore have been 350 cc. in the seven hours, or 75 cc. in an hour and a half, 0.5 per cent of which would be 0.38 gram of sodium chloride. This calculation is of course based on unjustified assumptions as to the constant rate of excretion of perspiration by different persons and its constant composition.

the bath, showed a considerable increase (15 to 50 per cent) in nitrogen and salt excretion as compared with the same period on days when no bath was taken, indicating that the bath had an immediate influence as well as a prolonged effect. A constant and uniform parallelism between nitrogen and chloride variations, noted also in earlier work, is not understood. Decreased perspiration through the cooling of the skin by the bath can account in only small measure for the greater salt and nitrogen excretion in the urine following the bath.

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RELATION BETWEEN THE AMOUNT OF CATALASE IN THE DIFFERENT MUSCLES OF THE BODY AND THE AMOUNT OF WORK DONE BY THESE MUSCLES

W. E. BURGE

From the Physiological Laboratory of the University of Illinois

Received for publication, June 2, 1916

Oxidase in plants and in animals has been the subject of much investigation. For a review of the literature on the subject see Oppenheimer, "Die Fermente und ihre Wirkungen," 1913 and Kastle, "Hygienic Laboratory Bulletin No. 59, 1909." The presence of catalase in the tissues has been demonstrated by many observers (1) employing a variety of reactions by which colored oxidation products are formed within the tissues. These observers (2) have shown that the capacity of the tissues to form these colored products has a direct relation to their capability of freeing oxygen from hydrogen peroxide, of bluing tincture of guaiac and of oxidizing salicylic aldehyde and benzyl alcohol to their respective acids. They found that there are definite and constant differences in the oxidative properties of the different tissues. This property was most marked in the spleen, liver and kidney and least marked in muscular and nervous tissue.

Although a great amount of work has been done in this field, at the same time, so far as I know, no one has attempted to correllate the amount of substance in a muscle that will liberate oxygen from hydrogen peroxide with the amount of external physical work done by the muscle. This substance will be referred to hereafter in this paper as catalase. The object of this investigation was to determine if there was more catalase in a muscle normally doing a relatively large amount of work than in one normally doing a relatively small amount of work, and if by increasing or decreasing the amount of work the amount of catalase would be correspondingly increased or decreased. The muscles of which the catalase content was to be determined were removed from the animals after the blood vessels had been washed free of blood by the use of large quantities of 0.9 per cent

sodium chloride and hashed in a hashing machine. The amount of oxygen liberated in ten minutes from 30 cc. of hydrogen preoxide by one gram of the hashed muscle was taken as a measure of the amount of catalase in the muscle. The solution of hydrogen peroxide used was prepared by diluting 15 cc. of commercial hydrogen peroxide to 30 cc. with distilled water.

Method. The apparatus used in this investigation in determining the amount of catalase is shown in figure 1; *a* and *a′* are two inverted burettes with their mouths beneath the water in a glass vessel *f; e* and *e′* rubber tubes connecting bottle *d* with the burettes; *b* and *b′* stopcocks and *c* a rubber stopper. The burettes were filled with water by sucking on *a* and *a′* and the stopcocks of the burettes closed. One gram of the hashed muscle was placed in a small crucible which was introduced into bottle *d*, rubber stopper *c* having been removed. Thirty cubic centimeters of the diluted hydrogen peroxide were introduced into the bottle care being taken that none of the hydrogen peroxide came in contact with the muscle at this time. Rubber stopper *c* was placed firmly in the mouth of the bottle while stopcocks *b* and *b′* were open. By turning the stopcocks of the burettes thus admitting air, the meniscus of the water in each burette was brought to the zero marking. Stopcock *b′* was then closed while *b* remained open. Bottle *d* was tilted and the contained crucible turned over thus mixing the hydrogen peroxide and the hashed muscle. The bottle was gently shaken for 10 minutes while the oxygen gas was being liberated and conducted by the rubber tube into the burette where it displaced the water. At the end of the ten minutes stopcock *b* was closed and the amount of gas read off directly from the burette. After making the necessary calculations for reducing the volume of the gas to standard

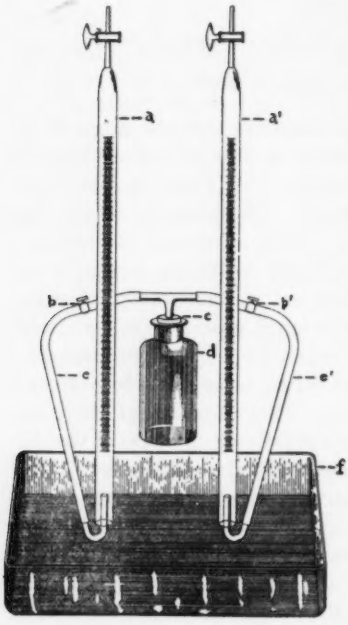

FIG. 1.

atmospheric pressure the resulting volume was taken as a measure of the amount of catalase in one gram of the muscles. The second burette a' was used when more than 100 cc. of oxygen was given off, each burette being of 100 cc. capacity. The temperature of the gas in the apparatus was kept fairly constant, 20°C., during the determinations.

In the manner described above determinations were made of the amounts of oxygen liberated in ten minutes from 30 cc. of hydrogen peroxide by the catalase in one gram of the hashed muscle of the heart and of the legs (biceps, gastrocnemius, semi-membranous, semi-tendinous) of dogs, rabbits, pigeons, rats, turtles and frogs; of the back (latissimus dorsi, trapezius serratus posterior inferior) of rabbits and rats; of the head (temporalis) of dogs and of the breast (pectoralis) of pigeons. These muscles will be referred to hereafter in this paper as heart, leg, back, head and breast muscles respectively.

TABLE 1

After heart, leg, and head are given the amounts of oxygen in cubic centimeters liberated in ten minutes from 30 cc. of hydrogen peroxide by the catalase in 1 gram of the respective muscles of dogs

DOG	1	2	3	4	AVERAGE
Heart	70	75	72	67	71
Leg	55	54	50	56	54
Head	32	39	33	31	35

Dogs. The four dogs used in the following experiments were tramp dogs obtained through the local dog catcher. The heart, leg and head muscles of these animals were washed free from blood and were then removed and ground up in a hashing machine. The amount of oxygen liberated in ten minutes from 30 cc. of hydrogen peroxide by the catalase in one gram of each of these groups of muscles was determined according to the method described. The results of these determinations are given in table 1. It may be seen that the average amounts of oxygen liberated by the heart muscle of the four dogs is 71 cc.; that by the leg muscles is 54 cc.; and that by the head muscles is 35 cc. It is assumed that the amount of catalase in these different groups of muscles is proportional to the amount of oxygen liberated, hence the amount of catalase in the leg muscles is 76 per cent of that in the heart and that in the head muscles 49 per cent. It would seem to be a fair assumption that of these muscles of the dog the heart under normal

conditions does the largest amount of work, the head muscles the least, and the leg muscles an intermediate amount, hence the amount of catalase in these muscles is proportional to the amount of work done.

Rabbits. These were born and raised in captivity. The heart, leg and back muscles were removed from these animals after the blood vessels had been washed free of blood with 0.9 per cent sodium chloride. They were then ground up in a hashing machine. The amounts of oxygen liberated in ten minutes by one gram of the different groups of muscles from 30 cc. of hydrogen peroxide is given after heart, leg and back in table 2. It may be seen that the average amount of oxygen liberated by the heart muscle of the four rabbits is 114 cc.; that by the leg muscles is 49 cc.; and that by the back muscles is 35 cc. Hence the amount of catalase in the leg muscles is 43 per cent of that in the heart and that in the back muscles is 30 per cent. As in the case of

TABLE 2

After heart, leg and back are given the amounts of oxygen in cubic centimeters liberated in ten minutes from 30 cc. of hydrogen peroxide by the catalase in 1 gram of the respective muscles of rabbits

RABBIT	1	2	3	4	AVERAGE
Heart	110	100	115	130	114
Leg	52	48	50	46	49
Back	40	36	38	31	35

the dogs the amount of catalase in the adult rabbits is greatest in the heart muscle, and intermediate in amount in the leg muscles.

Pigeons. The pigeons used in these experiments were ordinary male and female domestic pigeons accustomed to the range of the town. After the blood vessels had been washed free of blood with 0.9 per cent sodium chloride the heart, breast and leg muscles were removed and ground up. The catalase content of these muscles was determined as in the preceding experiments. The results of the determinations for four active male pigeons and four inactive sitting females are given in table 3. It may be seen that the heart muscle contained the largest amount of catalase, the breast muscles an intermediate amount, while the leg muscles contained the smallest amount. The high catalase content of the breast muscles is accounted for by the fact that normally these muscles do much work in the act of flying. The catalase content of the heart and breast muscles of the active male pigeons is

somewhat higher than that of the inactive sitting females. This may be accounted for by the fact that the muscles of the active males had been accustomed to do more work than those of the inactive sitting females.

From the preceding experiments it would seem that catalase is greatest in amount in muscles doing the greatest amount of work, least in amount in those doing least work and intermediate in amount in those doing an intermediate amount of work. It has been recognized for a long time that oxidation is more intense in muscles doing a great amount of work than in those doing a small amount of work and since catalase is greatest in amount in muscles doing the

TABLE 3

After heart, breast and leg are given the amounts of oxygen in cubic centimeters liberated in ten minutes from 30 cc. of hydrogen peroxide by the catalase in 1 gram of the respective muscles of active male pigeons, of inactive sitting females

Active males

PIGEON	1	2	3	4	AVERAGE
Heart	111	102	120	115	112
Breast	90	92	96	98	94
Leg	59	63	66	64	63

Inactive sitting females

Heart	92	98	109	104	101
Breast	87	86	89	93	89
Leg	52	60	58	62	58

largest amount of work and least in amount in muscles doing least work it follows that the amount of catalase varies with the intensity of the oxidation in the muscles.

Turtles and frogs. In table 4 under turtle and frogs are given the amounts of oxygen liberated by the heart and leg muscles of these animals. It may be seen that the average amount of oxygen liberated by the heart muscle of four turtles is 31 cc. and of the leg muscles 19 cc.; that of the leg muscles of the frogs from Chicago 18 cc., and that of the leg muscles of the frogs from New Orleans 22 cc. It may be recalled that the leg muscles of the dog, rabbit and active male pigeons liberated 54 cc., 49 cc., and 63 cc. of oxygen respectively and that the heart muscles liberated 71 cc., 114 cc. and 112 cc. If these amounts of oxygen be compared with those liberated by the corresponding mus-

cles of the turtle and frog it will be seen that they are much greater. It has been recognized for many years that oxidation in a warm blooded animal such as a dog is much more intense than in a cold blooded animal such as the frog. From these experiments it would appear that catalase is greater in amount in the muscles of warm blooded animals in which oxidation is more intense than in the muscles of cold blooded animals in which oxidation is less intense.

TABLE 4

After heart and leg are given the amounts of oxygen in cubic centimeters liberated in ten minutes from 30 cc. of hydrogen peroxide by the catalase in 1 gram of the respective muscles of turtles and frogs

Turtles from Chicago

TURTLE	1	2	3	4	AVERAGE
Heart	27	32	29	35	31
Leg	16	18	24	19	19

Small frogs from Chicago

FROG	1	2	3	4	AVERAGE
Leg	22	15	18	17	18

Jumbo bullfrogs from New Orleans

BULLFROG	1	2	3	4	AVERAGE
Leg	20	19	27	24	22

The following experiments were carried out to determine the effect of confinement on the catalase content of the heart, leg and breast muscles of male pigeons similar to those used in the preceding experiments. Nine pigeons were placed in a small cage so that there was no opportunity for the use of their breast muscles in flight. The catalase content of the heart, leg and breast muscles of three of these pigeons was determined before the period of confinement was begun. Similarly determinations were made at the end of the fifth, the tenth and the thirtieth days of confinement. The average amounts of oxygen liberated by the different groups of muscles of these pigeons are given after heart, leg and breast in table 5.

It will be observed that there had been little reduction in the catalase

content of the heart and leg muscles of the confined pigeons. The catalase content of the breast muscles, however, had fallen during the period of confinement as is indicated by the decrease in the amount of oxygen liberated from 89 cc. to 75 cc. after five days confinement, from 89 cc. to 59 cc. after ten days confinement and from 89 cc. to 58 cc. after thirty days confinement. The fact that the catalase content of the leg and heart muscles had decreased very little during confinement may be accounted for by the fact that these particular muscles were doing about the same amount of work during confinement as when the pigeons were at liberty. The physical work of the breast muscles of the pigeons, however, being reduced practically to zero during confinement was accompanied by a decrease in the catalase content of the muscle of approximately 35 per cent as is indicated by the decrease from 89 cc. to 58 cc. in the amount of oxygen liberated.

TABLE 5

After heart, breast and leg are given the amounts of oxygen in cubic centimeters liberated in ten minutes from 30 cc. of hydrogen peroxide by the catalase in 1 gram of the respective muscles of pigeons confined for 5, 10, and 30 days

PIGEON	0 DAYS CONFINEMENT	5 DAYS CONFINEMENT	10 DAYS CONFINEMENT	30 DAYS CONFINEMENT
Heart	109	104	103	101
Breast	89	75	59	58
Leg	57	56	57	54

The experiments now to be described were carried out to determine the effect of exercise on the catalase content of the heart, leg and back muscles of rats. Twelve ordinary barn rats, caught in traps, were placed in a small cage so that there was little opportunity for exercise. The catalase content of the heart, leg and back muscles of four of these rats was determined soon after they were caught. The average amount of oxygen liberated by one gram of the hashed muscles of these rats is given in table 6 after "Rats (unconfined)." The heart muscle liberated 109 cc. the leg muscles 38 cc., the back muscles 29 cc. The remaining rats were kept in a small cage for one month. At the end of this time the catalase content of the heart, leg and back muscles of four of these rats was determined. In the table after "Rats (confined)" is given the amounts of oxygen liberated by the heart, leg and back muscles of these rats. It may be seen that the catalase content of

all of these muscles had decreased as is indicated by a decrease in the amount of oxygen liberated.

If the effect of confinement on the catalase content of the muscle of the pigeons and of the rats be compared it will be found that but little change had been produced in the catalase content of the hearts of these animals by confinement; that the catalase content of the muscles of the legs of the pigeons was not changed while it was decreased in the muscles of the legs of the rats. This difference in effect is

TABLE 6

After heart, leg and back are given the amounts of oxygen in cubic centimeters liberated in ten minutes from 30 cc. of hydrogen peroxide by the catalse in 1 gram of the respective muscles of active unconfined rats, inactive confined rats and rats exercised

RAT	1	2	3	4	AVERAGE
Rats unconfined					
Heart	110	100	120	106	109
Leg	36	34	39	42	38
Back	25	28	32	30	29
Rats confined					
Heart	96	93	99	100	97
Leg	30	32	28	34	31
Back	26	20	30	24	25
Rats exercised					
Heart	104	96	101	108	102
Leg	38	40	46	44	42
Back	31	34	28	29	30

accounted for by the fact that the physical work of the muscles of the legs of the pigeons was about the same during confinement as when the pigeons were at liberty while the physical work of the muscles of the legs of the rats was reduced during confinement with a corresponding reduction in the catalase content of the muscles.

The four remaining rats that had been confined in a small cage for a month were given exercise in the following manner. An ordinary circular cheese box 35 cm. in diameter was attached to a motor reducing gear. A piece of circular wire netting with door cut in it was used as a top for the box. The box was adjusted so that it could be rotated

with its vertical axis in an horizontal direction. The rats were introduced into the box and it was rotated at a speed of fourteen revolutions per minute six hours each day, three hours in the morning and three hours in the afternoon for six successive days. This speed of rotation kept the rats walking in a comfortable manner. At the end of the six days the catalase content of the heart, leg and back muscles of the animals was determined. In table 6 after "Rats (exercised)" is given the amount of oxygen liberated by the respective muscles. It may be seen that the average amount of oxygen liberated by the heart muscle was 102 cc., that by the leg muscles 42 cc.; that by the back muscles 30 cc. By comparing these amounts of oxygen with those liberated by the corresponding muscles of "Rats (confined)" it may be seen that exercise had increased the amount of catalase in the leg and back muscles as is indicated by the increase in the oxygen liberated while little change had been produced in the catalase content of the heart muscle.

It is recognized that oxidation can be increased or decreased in a muscle by increasing or decreasing the amount of work done by the muscle. It may be seen in the preceding experiments with pigeons and rats that when the work of the muscles was increased with the resulting increase in oxidation the amount of catalase was increased and vice versa.

CONCLUSIONS

1. The amount of catalase in the different muscles of the body varies with the amount of work done by these muscles; those doing the greatest amount of work contain most catalase, while the muscles doing the least work contain least catalase.

2. By increasing or decreasing the external physical work of a muscle the amount of catalase is correspondingly increased or decreased. If the external physical work of a muscle such as the pectoralis of pigeons is reduced practically to zero the amount of catalase is reduced by approximately 38 per cent.

3. Catalase is greater in amount in the muscles of warm blooded animals in which oxidation is more intense than in corresponding muscles of cold blooded animals in which oxidation is less intense.

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A FURTHER STUDY OF THE BLOOD GASES DURING HIBERNATION IN THE WOODCHUCK (MARMOTA MONAX) THE RESPIRATORY CAPACITY OF THE BLOOD

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Received for publication, June 6, 1916

INTRODUCTION

In a former paper (1) it was shown that during hibernation of the woodchuck there is an increase of CO_2 in the blood from about 70 volumetric per cent to as high as 100 volumetric per cent in the case of the venous blood, and from about 68 volumetric per cent to as high as 87 volumetric per cent in the arterial blood. This confirmed the observations of Dubois on the European marmot. In discussing the results it was recognized that this difference in the blood of the two states might have been exaggerated to some extent because of the use of ether in immobilizing the active animal, while no anaesthetic was used on the dormant ones. It was concluded from a single case in which chloretone was used that the probable error was slight. In order to make sure of this point, it was finally decided to make a more extensive series of observations along a similar line except that some other means be used to immobilize the active animal.

A saturated aqueous solution of chloretone was found to be very slow in its action and to involve the injection of too large a quantity of water to be absorbed and thus tend to dilute the blood. An alcoholic solution was tried and found to be more satisfactory, especially when aided by cocaine as a local anaesthetic, if given in a large enough dose. Due, however, to the death of about fifteen woodchucks from some unknown cause, the number of animals available for this experiment was unintentionally limited.

It was also mentioned in the paper referred to above that DuBois (2) concluded that the respiratory capacity of the blood was greater during hibernation because of an increase in the concentration of the blood. He found that defibrinated blood obtained from a hibernating

marmot with a rectal temperature of 8.6°C. when treated for one hour at 12°C. with a current of air and CO_2, contained 124.7 volumetric per cent CO_2, 18.6 volumetric per cent O_2 and 2.9 volumetric per cent N. The same treatment in summer in the case of the blood of an active animal with a rectal temperature of 34.2°C. yielded only 103.55 volumetric per cent CO , 13.06 volumetric per cent O_2 and 2.6 volumetric per cent N. Somewhat similar figures to these latter were obtained from an animal which while dormant in the winter had been bled and allowed to slowly warm up at the rate of 2.6°C. in four hours, and which was bled a second time when the marmot had a rectal temperature of 35.4°C. The blood obtained at this second bleeding and treated in the same way had less oxygen in it than the blood of the active animal, otherwise it was very similar. This indicated that the cause of the increased respiratory capacity of the blood during winter-sleep was due to its increased concentration. To strengthen this belief was the fact that the specific gravity of the non-defibrinated arterial blood of a torpid marmot was 1.071 while that of the defibrinated was 1.0654 as compared with 1.046 for non-defibrinated and 1.0525 for defibrinated arterial blood of an active animal.

From an examination of the specific gravity of the blood of a few woodchucks, some of which were killed during the active state and others during hibernation, it appeared that no such striking increase in the specific gravity of the blood occurred in these animals. It was, therefore, interesting to know if the respiratory capacity of the blood similarly showed no increase, granting that the conclusions of Dubois were correct. A series of experiments were carried out to test this point and to see the relationship of the respiratory capacity of the blood to its actual oxygen and carbon-dioxide content in both the active and the dormant state.

METHOD

One hour before the operation of exposing the blood vessels commenced, the animal (whether torpid or active) was given per rectum for each kilogram of body weight 2 cc. of 50 per cent alcohol containing 0.3 gram of chloretone, which is twice the dose prescribed for the rabbit. This gradually immobilized the active animal without excitation, and did not disturb the hibernating ones. With the additional use of a little cocaine as a local anaesthetic, the operation of exposing the vessels could be done without exciting the animal.

As a rule the hibernating animals of this series were not as dormant

as is the general rule. Since the carotid artery, which is much more inaccessible especially in the woodchuck on account of such a short neck, was used instead of the femoral artery, the chloretone treatment during winter-sleep served to lessen the possibilities of disturbing the animal during the operation. Thus as many factors as practicable were kept constant throughout the whole series.

The apparatus used and the further procedure were essentially the same as that described in the previous paper, except that only three manometers were used on each sample of blood. The apparatus being in better working order and the manipulations having become more accurate as a result of increased experience, a larger number of duplicate analyses were not necessary. The venous blood was collected by means of an ordinary cannula from a large deep branch of the jugular, thus doing away with the necessity of the T tube used as a cannula in the previous series.

About 2 cc. of blood of each kind (venous and arterial) was drawn into two sampling pipettes. Then nearly 6 cc. of arterial blood was collected in a specific gravity bottle, and finally 20 cc. of arterial blood was collected and defibrinated in a small flask containing glass beads. This defibrinated blood was filtered through absorbent cotton and divided into two equal portions. Through one portion was passed, for one hour at a temperature of 20°C., a steady stream of oxygen which had just been washed in water. The other portion was treated similarly, for one hour also at 20°C., with a stream of carbon dioxide which had just been passed through a solution of sodium bicarbonate. The blood being saturated with these gases was kept in closed vessels during the process and the rate of the flow of gas was kept as nearly equal as practicable throughout the whole series. Whatever evaporation or dilution that might result during this process ought to be fairly uniform.

While the defibrinated blood was being saturated with gases, the first samples of blood taken were analysed for oxygen. Then the two samples of defibrinated blood, which had by this time become saturated, were analysed for oxygen. Finally the analysis of all the samples for carbon dioxide was carried out together. If care was taken in handling the defibrinated blood which had been saturated with carbon dioxide so as to expose it to the air only momentarily and then to quickly take a sample from the bottom and unexposed portion, such blood contained no oxygen, and similarly the blood saturated with oxygen contained no carbon dioxide. These items, consequently, do not appear in the tables of results which follow.

RESULTS

The results are tabulated in the three accompanying tables. The data as seen in the first two tables below confirm in general those obtained in the earlier series, and show that the amount of CO_2 normally present in the active woodchuck is comparatively high, being nearly

TABLE 1

Blood gases before hibernation

ANIMAL (SERIES III)	DATE OF ANALYSIS	GAS IN CC. PER 100 CC. OF BLOOD REDUCED TO 0°C. AND 760 MM. PRESSURE			CONDITION OF ANIMAL		
		A = Arterial V = Venous D = Difference	O_2	CO_2	Rectal temperature	Reduced body weight	Remarks
					deg. C.	*grams*	
32	October 14, 1915	A	19.91	64.85	37.3	3,504	Active
		V	16.29	75.16			
		D	3.62	10.31			
33	October 19, 1915	A	19.40	77.55	36.7	1,830	Active
		V	16.00	86.30			
		D	3.40	8.75			
34	October 23, 1915	A	19.41	78.62	35.6	1,925	Active
		V	13.26	85.70			
		D	6.15	7.08			
35	October 26, 1915	A	23.21	66.62	37.1	2,400	Active
		V	16.64	74.23			
		D	6.57	7.61			
36	October 30, 1915	A	20.84	62.95	34.7	1,900	Active
		V	10.19	77.51			
		D	10.65	14.56			
38	November 13, 1915	A	19.47	70.61	33.9	2,688	Active
		V	16.02	75.99			
		D	3.45	5.38			
41	December 4, 1915	A	16.80	67.00	34.0	1,258	Active
		V	10.80	72.51			
		D	6.00	5.51			
Average.....		A	19.86	69.75	35.2	2,215	
		V	14.17	78.20			
		D	5.69	8.46			
Average of active animals of the earlier series where ether was used		A	19.07	67.86	31.7		
		V	13.04	70.08			
		D	6.03	2.22			

TABLE 2

Blood gases during hibernation

ANIMAL (SERIES III)	DATE OF ANALYSIS	GAS IN CC. PER 100 CC. OF BLOOD REDUCED TO 0°C. AND 760 MM. PRESSURE			CONDITION OF ANIMAL		
		A = Arterial V = Venous D = Difference	O_2	CO_2	Rectal temperature	Reduced body weight	Remarks
					deg. C.	*grams*	
44	December 23 1915	A	22.69	68.87	13	3,051	Dormant. Had a rectal temperature of 9°C. five days previously.
		V	7.18	90.34			
		D	15.51	21.47			
45	December 28 1915	A	20.50	79.13	10	2,046	Dormant
		V	15.33	94.01			
		D	5.17	14.88			
46	February 1 1916	A	17.44	60.74	9	1,780	Dormant. All but one other animal in these burrows were awake and active
		V	12.07	68.96			
		D	5.37	8.22			
47	February 3 1916	A	19.36	65.52	6	1,580	Dormant. Also asleep two days previously. Other animals in the same burrow awake with rect. T. 35°C.
		V	9.68	81.63			
		D	9.68	16.11			
52	March 4 1916	A	20.51	84.97	10	1,203	Very dormant
		V	19.70	99.75			
		D	.81	14.78			
53	March 6 1916	A	25.38	87.99	12	1,849	Very dormant
		V	13.30	94.52			
		D	12.08	6.53			
Average.....		A	20.98	74.51	8.6	1,918	
		V	12.88	88.20			
		D	8.10	13.69			

70 volumetric per cent as an average of seven animals. This is slightly less than 2 volumetric per cent higher than was obtained when ether was used—the average of the six determinations made with ether as the anaesthesia being placed at the end of table 1 for ready comparison.

All other figures also compare well, the variations being as close as could be expected from the limited number of animals involved, except the lower percentage of CO_2 in the venous blood of the ether series.

There is on an average only a difference of 2.22 volumetric per cent in the CO_2 in the venous and in the arterial blood where ether was used, as compared with 8.46 volumetric per cent difference in the chloretone series. This error, while somewhat great when taken alone, does not seriously modify the general difference observed between the gases in the blood of the two states.

By comparing tables 1 and 2 it will be seen that the CO_2 content of the blood is much higher during hibernation, reaching nearly 100 volumetric per cent in animal 52. The average per cent of CO_2 in the blood of this series of six hibernating animals is probably not representative because of animals 46 and 47. During the latter part of January and early part of February all our woodchucks, except these two (46 and 47) were awake and more or less active, some of them having a rectal temperature of 35°C. Since it was desirable to get analyses during the middle part of the hibernating period, the only two animals that were dormant were utilized. These two woodchucks were freely accessible to the active ones since the burrows communicated, and had undoubtedly been disturbed. It has been repeatedly shown that when hibernating animals are disturbed and awakening commences, there is a great increase in the elimination of CO_2 (3). In general the duration and depth of torpor obtained during this last winter was unmistakably much less than that obtained during the preceding season. The unusually mild weather which existed for a long period in January naturally suggests itself as having had something to do with this unusually long period of awakening, although the relation of cold to winter-sleep is not agreed upon.

The fact that the two woodchucks under consideration were asleep while there was practically no excess of CO_2 in the blood, has a bearing upon the theory of Dubois, namely that the accumulation of an excessive amount of CO_2 in the blood and tissues of hibernating animals is an essential condition in maintaining dormancy during winter sleep. Here are apparently two cases of torpor with practically no excess of CO_2, unless these particular animals in the active state naturally had much less CO_2 in the blood than has been the case in any woodchuck so far investigated. There is, of course, always this possibility. Then again there is the possibility that the excess of CO_2 in the system may only be a result of altered ventilation and a decrease in the irritability of the respiratory center. The air in the burrows must become heavily charged with CO_2 because of the comparatively small amount of air confined within when the snow covers the ground. If the respiratory

center during this period is less sensitive to the increased H ion concentration which would follow the higher CO_2 pressure in the alveolar air, CO_2 would naturally accumulate in the blood and tissues.

A further comparison of tables 1 and 2 shows that there is distinctly a greater difference between the amount of CO_2 in the venous and in the arterial blood of the hibernating animal. Similarly, there is a greater difference between the amount of O_2 in the arterial and in the venous blood during winter sleep.

In the previous paper, it was stated that a number of observers (Valentin, Quincke, Claude Bernard, Marès, Allen Cleghorn) had noted that the blood of hibernating animals was unusually bright in the veins, leading some of them to conclude that all the blood was arterial during winter-sleep. In animal 52 there is nearly as much O_2 in the venous blood as in the arterial. No difference in color could be detected after being collected in the sampling pipettes. This is the only case where in an undisturbed animal such a condition was observed. It may be noted, however, that the maximum percentage of O_2 recorded in any case where there was no evidence of overventilation in either series was found during hibernation. In the ether series it amounted to 25.76 volumetric per cent in the arterial blood of a torpid animal (rectal temperature 6°C., no anaesthesia) examined December 19, 1914. In the chloretone series it occured also in the arterial blood of a dormant animal (rectal temperature 12°C., chloretone) examined March 6, 1916, and amounted to 25.38 volumetric per cent.

The results of saturating the defibrinated arterial blood with CO_2 and O_2 are found in the table below in connection with other related data.

The table shows that during hibernation there is in the woodchuck no difference in the O_2 absorbing capacity of the blood, but a distinct decrease in the power to absorb CO_2, notwithstanding the fact that the average specific gravity of the blood of these particular dormant animals is slightly greater than that of the active ones. The average weight of the active animals is but slightly greater than that of the torpid ones, hence this difference is not due to having used smaller animals during the winter, so that the withdrawal of the same amount of blood would represent in the smaller hibernating animal a relatively greater hemorrhage and cause a greater dilution of the blood by the influx of lymph as the blood pressure falls. It may also be mentioned here that during winter-sleep the blood volume probably decreases slightly less rapidly than does the body weight as a whole, at least the

percentage of blood does not decrease below that found just before hibernation. The data supporting this statement will be published later. Hence the blood volume of the hibernating animals used here is undoubtedly fully equal to that of the active ones.

TABLE 3

Respiratory capacity of the blood

ANIMAL (SERIES III)	DATE OF ANALYSIS	GAS IN CC. PER 100 CC. OF DEFIBRINATED BLOOD REDUCED TO 0°C AND 750 MM. PRESSURE		SPECIFIC GRAVITY OF BLOOD	CONDITION OF ANIMAL		Remarks
		O_2	CO_2		Rect. temp.	Reduced body weight	
					deg C.	*grams*	
Before hibernation							
33	October 19, 1915.....	20.88	222.48	1.0573	36.7	1830	Active
34	October 23, 1915.....	24.42	247.34	1.0579	35.6	1925	Active
35	October 26, 1915.....	24.03	235.46	1.0646	37.1	2400	Active
36	October 30, 1915.....	21.16	212.69	1.0577	34.7	1900	Active
38	November 13, 1915....	24.88	205.12	1.0596	33.9	2688	Active
41	December 4, 1915.....	16.14	216.40	1.0562	34.0	1258	Active
Average		21.92	223.25	1.0589	35.3	2000	
During hibernation							
44	December 23, 1915.....	22.39	215.79	1.0620	13	3051	Dormant
45	December 28, 1915.....	24.86	197.81	1.0647	10	2046	Dormant
46	February 1, 1916.....	19.13	189.21	1.0571	9	1780	Dormant
47	February 3, 1916.....	21.18	203.79	1.0631	6	1580	Dormant
52	March 4, 1916.....	20.18	217.26	1.0565	10	1203	Dormant
53	March 6, 1916.....	23.92	231.88	1.0628	12	1849	Dormant
Average		21.94	209.29	1.0610	10	1918	

The results show also that the blood of the woodchuck has an extraordinary capacity for absorbing CO_2. In the six active animals it ranges from 205.12 volumetric per cent to 247.35 volumetric per cent with an average of 223.25 volumetric per cent. In the six hibernating animals the limits are 189.21 volumetric per cent and 231.88 with an average of only 209.29 volumetric per cent. Dubois maintains that the respiratory capacity of the blood increases during winter-sleep in the case of the European marmot. His investigation was, however, limited to

three animals and in these the variation in the specific gravity of the blood was far greater than has ever been found in the woodchuck in this laboratory, where up to the present time the specific gravity of the blood of about 50 woodchucks has been determined.

In view of the fact that during inanition, especially of man and other animals whose food is partly or entirely of a vegetable character, there is a decrease in the alkalinity of the blood, i.e., a tendency towards acidosis as deducted from the analysis of the urine and alveolar air (4), one would expect the amount of CO_2 capable of being absorbed by the blood to be distinctly less during hibernation, of herbivorous animals at least. The woodchuck is strictly herbivorous, but during winter-sleep it lives entirely on its own tissues and so may be considered a carnivor. Of the tissues consumed during hibernation a very large fraction is adipose. The respiratory quotient is, therefore, small during lethargy. It has been shown by several authors (5) that the respiratory quotient may fall as low as 0.5, thus indicating not only that the food is fat and proteins, but also that there is a retention of oxygen, undoubtedly as CO_2 and probably also in the form of some incompletely oxidized products. A fat-rich diet is now looked upon as tending to produce acidosis because of incomplete oxidation of the fats and consequently the formation of acetone bodies (acetone, aceto-acetic, B-oxybutyric acid). (6)

That the withdrawal of the alkalies normally supplied in a vegetable diet and utilization of purely animal matter (the tissues of the body) as occur with the onset of hibernation, does increase the acid radicals (other than CO_2) relative to the basic radicals in some hibernating animals is in general supported by the meagre facts available on the reaction of the urine of such animals. Thus Dubois (7) found invariably that the urine of the marmot during the summer when fed on its normal vegetable diet was alkaline, while that of the dormant animal was acid. From Polimanti's (8) review of the literature there is some disagreement, however. Horvath reports that the spermophile voids a large quantity of alkaline urine when first it wakes up from winter sleep. Albini found that the urine discharged by the marmot when it wakes is neutral, while Quincke's examination of the urine of a hibernating marmot indicated a slightly acid reaction, with some albumin and skatole, but no indican. I. Monti explained the difference in the reaction of the urine upon a difference in the amount of gastric juice in the stomach. Polimanti's own observations (p. 351) are in agreement with those of Dubois on the alkaline character of the marmot's urine

in the active state while being fed its usual vegetable diet. He agrees also with Dubois and Quincke on the acid reaction of the urine during hibernation. While nothing has been done on the urine of the woodchuck, there is every reason to believe that the conditions are the same as in the European marmot. The figures given by Noe (9) on the acidity of the urine of the hedgehog (an insectivore), excluding the single case reported in June, show a low acidity in January and an increase in acidity with the progress of winter, reaching a maximum in March (corresponding probably to the end of hibernation) after which there is a decrease again.

It is well known that weak acids readily drive out the CO_2 from the blood. It was, for example, shown by Walter (10) many years ago that the introduction of hydrochloric acid into the stomach of the rabbit may reduce the CO_2 in the blood to only 2 or 3 volumetric per cent. It is but to be expected that during hibernation of the woodchuck there will be sufficient acidosis to distinctly decrease the power of the blood to absorb CO_2. The fact that the blood actually contains more CO_2 during the dormant period would indicate that the respiratory center has become much less sensitive to the H ion concentration of the blood and that the CO_2 pressure in the alveoli of the animal must also be very high in order to maintain such a large amount of CO_2 in the blood in the presence of a probable reduction in its alkalinity.

It is strange that the arterial blood does not contain less oxygen during hibernation than during the active state, if such an increase in the H ion concentration actually occurs. Following the discovery by Bohr, Hasselbalch and Krogh (11), that an increase in the amount of CO_2 in the blood increases the relative amount of reduced hemoglobin, it was found that other acids also decreased the effective concentration of oxygen and that the effect is due to the H ion concentration (12). The results above show, however, that there is, if any thing, more oxygen in the arterial blood during hibernation than during the active state.

SUMMARY

1. A determination of the blood gases after treating woodchucks with chloretone, indicates that the use of ether in the previous series of experiments on blood gases during hibernation decreased somewhat the amount of CO_2 in the venous blood of the active animal.

2. All other figures in this series agree with those obtained in the first series as closely as could be expected, and hence show the same general

condition—that there is a relatively high CO_2 content in the blood of the woodchuck even when awake and active, that there is a marked increase in the amount of CO_2 in the blood during hibernation and especially during the latter part of this torpid state, and that the difference in the amount of gases in the venous and in the arterial blood is greater during winter-sleep than during the active state.

3. A single case was found in which during hibernation the venous blood could not be distinguished from the arterial by its general appearance. This venous blood upon analysis contained only 0.81 volumetric per cent less oxygen than did the arterial. This condition, which has been reported as being the general rule in the dormant state of hibernating animals by a number of observers, is only rarely found in the woodchuck.

4. The oxygen absorbing power of the blood is not changed during hibernation.

5. The absorbing power of the blood for CO_2 decreases during hibernation This may be ascribed to a probable decrease in the alkalinity of the blood.

Again the encouragement and direction received from Dr. Sutherland Simpson during the progress of this investigation is acknowledged, as is also the assistance rendered by Gertrude B. Rasmussen during the operations.

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THE DUCTLESS GLANDS AND HIBERNATION

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Received for publication June 8, 1916

The phenomenon of hibernation is of great interest to the biologist. Its possible causes and physiologic processes have been the subject of many investigations. These experiments and observations have demonstrated many facts in regard to hibernation, and have especially emphasized the profound change in metabolism which takes place in the hibernating animal.

Many of the ductless glands certainly have considerable to do with the metabolic processes. As the changes in metabolism are so marked during the hibernating state, it was thought that possibly new insight into the function of some of the ductless glands, or a cause for hibernation, might be obtained by a study of these glands in a hibernating animal. Observations were carried out during the summer of 1914 and the winter of 1914–15, on a series of spermophilus tridecemlineatus (gophers). The number of animals studied was small and the results inconclusive. The publication of positive results by other workers (1) incited me to repeat the experiment, using a larger series of animals and observing control conditions more carefully. I shall report the complete study.

While many studies have been undertaken on different phases of hibernation (2) very few have been made with special reference to any of the ductless glands.

Gemelli (3) made a study of the hypophysis in 22 adult marmots. Some of these animals were sacrificed while in the hibernating state; others shortly after awakening, and the rest during the summer season. Each hypophysis was studied histologically. Gemelli did not find any modification in the nerve-lobe or in the posterior part of the glandular lobe. In the anterior part of the glandular lobe there were marked changes. These occurred only in the chromophile cells. During the summer these cells were accumulated in big clumps, but during hibernation they were enormously decreased. Shortly after the animal awakened many mitotic figures were found.

After our study was begun, Cushing and Goetsch (1) published an article dealing with the subject. Beside a general review of the clinical and experimental work done on the pituitary gland, the data submitted consisted of an histologic study of most of the ductless glands of seven woodchucks, three of which were hibernating and four active. The observations showed evidence of pluriglandular insufficiency in the dormant state, but the most striking change occurred in the anterior lobe of the pituitary gland. The latter consisted of a loss of characteristic cellular topography of the pars anterior, and, in the case of the individual cells, of a shrinkage of both the nuclear and protoplasmic substance of the cell with complete loss of the characteristic histologic picture of the active gland, namely, the differential staining qualities of the granular content with acid and basic dyes. On the basis of these observations Cushing and Goetsch (1) conclude that hibernation may be ascribed to the seasonal physiologic wave of pluriglandular inactivity. The essential role may perhaps be ascribed to the pituitary body not only because the most striking histologic change appears in this structure but also because deprivation of the secretion of this gland alone, of the entire ductless gland series, produces a group of symptoms comparable to hibernation.

In our experiments the spermophile was selected because of its great prevalence locally. While it is probably not as typical an example of the hibernating animal as the marmot, it does become profoundly lethargic. It was found to be a very satisfactory laboratory animal, being easily cared for and free from disease. However, several facts must be considered in adapting it to laboratory use. The old animals withstand operation very well but are prone to tear open their own or another's wound. To avoid this they must be kept in separate cages for a few days after operation and the wound should be sponged with a strong solution of iodin. The young during the first three or four months of life do not withstand operation very well. As the spermophiles do not all hibernate at the same temperature, it is advisable to maintain the temperature of the room in which several animals are kept together in one cage above the hibernating temperature; otherwise the active animals will kill those that are torpid. During the season of rut it is necessary to keep the males separate.

While the causative processes of hibernation are still unknown, there are environmental conditions which have long been considered important factors, namely, lowered temperature and a decreased food supply. To these may be added an intrinsic factor, the nutritional

condition of the animal. I attempted to control these factors but found that in the spermophile they are of importance only within very wide limits. A spermophile will not become torpid immediately after eating, and withdrawal of food seems to be a factor in producing torpidity. However, it will hibernate even when it has access to a good food supply if the other factors are favorable. While changes of temperature are of the greatest importance, they are active only within a wide limit. Very rarely will an animal become torpid at a temperature higher than 24°C. and usually every animal will be lethargic when the temperature gets as low as 5°C. An animal does not always hibernate at the same temperature. It may be torpid at a cer-

TABLE 1

*The temperature at which 100 spermophiles first became torpid**

CONDITION OF ANIMAL	TOTAL NUMBER	TEMPERATURE (CENTIGRADE)					
		15°		20°	10°	15°	5°	10°
		Number becoming torpid	Percentage of total	Number becoming torpid	Percentage of total	Number becoming torpid	Percentage of total
Normal	60	40	66⅔	11	18⅓	9	15
Ovaries removed	10	6	60	1	10	3	30
Testicles removed	10	6	60	2	20	2	20
Adrenals removed	10	5	50	3	30	2	20
Thyroids removed	10	6	60	2	20	2	20

* Note that the removal of the ovaries, testicles, adrenals, and thyroids did not materially change the percentage of those becoming torpid at the different temperatures as compared with normal animals.

tain temperature and a few days later active at a much lower temperature. Some animals after once becoming torpid remain in that condition for months. Others after becoming torpid may at various times be warm to the touch though not active. Still others pass through periods of torpidity and periods of activity when they will take food, without any apparent relation to temperature. In general, a very fat animal and a very thin one will become torpid sooner than an animal that is very active. It is thus seen that while temperature, food and the nutritional conditions are important factors, they are of value only when taken in consideration with the general phenomena of hibernation. In our study the temperature was found to be the only factor of any practical importance and it was of significance only when carefully controlled.

The animals were kept in several rooms so that each experiment could be carefully checked in regard to laboratory conditions. Some of the rooms were dark and could be maintained at any desired temperature. Other rooms were kept very warm, and others were allowed to change with the outside temperature. Usually when it was desired to make an animal hibernate, food was withdrawn, the animal was placed in a dark room and the temperature was gradually decreased. This approximated normal conditions, and as a rule, the method proved to be very satisfactory.

Two general methods were employed in investigating the possible relationship between the ductless glands and hibernation.

The first method consisted in a comparative histologic study of all the glands in active and torpid animals. The active animals were sacrificed by bleeding under light ether anesthesia. Specimens from active animals were obtained every few weeks throughout the year, thus eliminating the effect of seasonal variation. The glands of the hibernating animals were obtained at various times after the beginning of the lethargic condition. The torpid animals were sacrificed by bleeding; it was not necessary to anesthetize them. The specimens were fixed mainly in formalin, acetic Zenker and neutral formalin Zenker.

It is obvious that if any of the ductless glands are a causative factor in hibernation, only initial changes can be considered as offering any valid data in a study of this character, because after a long-continued period of hibernation, the changes might be the result and not the cause of the torpid state. For this reason the greatest attention was paid to the animals which had hibernated only a short time.

The second method consisted in removing certain of the ductless glands, and after various lengths of time determining whether the operated animals would become torpid in the same manner as the unoperated controls. If any of the glands of internal secretion are causative factors or inhibit the hibernating state, removal of these glands should vary the normal induction of torpidity. Of course this method could be used only in a study of the glands which can be surgically removed. Practically it was found that the sex glands, thyroids and adrenals were the only glands that could be studied by this method.

A study of all the tissues of the hibernating spermophile showed that there is one slight change which in general is common to most tissues. This change consists in a slight shrinking of the cell, with a decrease in both the distinctness of the cell outline and the intensity

of the staining reaction. In additiion, there are marked fatty changes, especially in the liver, and congestion, especially in the spleen.

The ductless glands of the spermophile are very small. Because of this fact, several were lost or destroyed during the process of histologic technic. However, a sufficient number were obtained for a complete study.

The sex glands. With regard to hibernation there are minor differences between the male and female. In the wild state it seems that, in general, the male is the first to appear in the spring and the last to disappear in the fall. My observations seem to show that the female becomes torpid sooner than the male. The difference, however, is very slight.

The seasonal variation in the sex glands and accessory reproductive organs is well marked but need not concern us here except in regard to the fact that it takes place in animals that have been active all winter a little sooner than in those that have hibernated. Also as far as could be determined, captivity does not interfere with the sexual cycle except that it causes it to begin and terminate earlier in the season.

The testicle. The examination of the testicles at various periods of the year showed the changes occurring at various stages of the sexual cycle. These changes are very slight from the beginning of early fall to the beginning of rut.

In July, 1914, the testicles were removed from two spermophiles. The animals hibernated under exactly the same conditions as the normal controls during the winter of 1914–15. One of them was observed also during the fall of 1915 and no change was noted.

During the summer of 1915, twelve animals were castrated. These animals hibernated normally.

The ovaries. The ovaries show a marked seasonal variation. The luteal cells do not persist until the beginning of hibernation. The changes occurring in the ovary are those incident to the development of a large number of graafian follicles. Most of these follicles are developed before the period of hibernation, and during hibernation the changes are slight.

The ovaries were removed from two spermophiles in July, 1914. The animals hibernated normally during the winter of 1914–15 and the fall of 1915. During the summer of 1915 the ovaries were removed from twelve animals. All hibernated under the same condition as the normal animals.

TABLE 2

The effect of decrease in temperature on a series of normal and operated spermophiles

DATE	TIME OBSERVED A.M.	NORMAL ADULT MALE	NORMAL ADULT MALE	NORMAL ADULT FEMALE	NORMAL ADULT FEMALE	NORMAL YOUNG MALE	NORMAL YOUNG FEMALE	ADULT TESTICLES REMOVED	YOUNG TESTICLES REMOVED	YOUNG TESTICLES REMOVED	YOUNG TESTICLES REMOVED	ADULT OVARIES REMOVED	ROOM TEM. C.
11–11–15	8.00	active	active	active	active	active	active	active	active	active	active	active	25
11–12–15	8.00	active	sltly. torpid	active	active	active	active	active	active	active	active	active	20
11–13–15	8.00	active	torpid	active	torpid	active	active	active	active	active	active	sltly. torpid	19
11–14–15	9.00	active	torpid	sltly. torpid	torpid	torpid	torpid	sltly. torpid	sltly. torpid	active	active	torpid	18
11–15–15	8.00	active	torpid	sltly. torpid	torpid	torpid	torpid	sltly. torpid	torpid	active	active	torpid	17
11–16–15	8.00	active	torpid	sltly. torpid	torpid	torpid	torpid	sltly. torpid	torpid	sltly. torpid	active	torpid	16
11–17–15	8.00	active	torpid	torpid	torpid	torpid	torpid	sltly. torpid	torpid	sltly. torpid	active	torpid	15
11–18–15	8.00	active	torpid	torpid	torpid	torpid	torpid	torpid	torpid	torpid	active	torpid	10
11–19–15	8.00	active	torpid	torpid	torpid	torpid	torpid	torpid	torpid	torpid	active	torpid	10
11–20–15	8.00	torpid	torpid	torpid	torpid	torpid	torpid	torpid	torpid	torpid	sltly. torpid	torpid	5

DATE	TIME OBSERVED A.M.	ADULT OVARIES REMOVED	ADULT OVARIES REMOVED	ADULT OVARIES REMOVED	ADULT FEMALE, THYROID REMOVED	ADULT FEMALE, THYROID REMOVED	ADULT FEMALE, THYROID REMOVED	ADULT FEMALE, THYROID REMOVED	ADULT FEMALE, ADRENALS REMOVED	ADULT FEMALE, ADRENALS REMOVED	ADULT FEMALE, ADRENA. REMOVED	ADULT FEMALE, ADRENALS REMOVED	ROOM TEM. C.
11–11–15	8.00	active	active	active	active	active	active	active	active	active	active	active	25
11–12–15	8.00	active	active	active	active	active	active	active	active	active	active	active	20
11–13–15	8.00	sltly. torpid	active	active	active	active	active	active	active	active	active	sltly. torpid	19
11–14–15	9.00	sltly. torpid	torpid	active	sltly. torpid	sltly. torpid	sltly. torpid	sltly. torpid	active	sltly. torpid	active	sltly. torpid	18
11–15–15	8.00	torpid	torpid	active	sltly. torpid	torpid	torpid	sltly. torpid	active	sltly. torpid	active	sltly. torpid	17
11–16–15	8.00	torpid	torpid	active	active	torpid	torpid	sltly. torpid	active	sltly. torpid	active	torpid	16
11–17–15	8.00	torpid	sltly. torpid	active	active	sltly. torpid	sltly. torpid	sltly. torpid	active	sltly. torpid	sltly. torpid	torpid	15
11–18–15	8.00	torpid	torpid	active	torpid	sltly. torpid	torpid	torpid	active	torpid	torpid	torpid	10
11–19–15	8.00	torpid	torpid	torpid	torpid	torpid	torpid	torpid	active	torpid	torpid	torpid	10
11–20–15	8.00	torpid	torpid	torpid	torpid	torpid	torpid	torpid	sltly. torpid	torpid	torpid	torpid	5

The thyroids. The histologic picture of the thyroid of the spermophile is very uniform. The acini are relatively large and filled with colloid; the cells very low or flat. The secretion granules are usually very few in number. I have not noticed any difference between the thyroids of active and hibernating animals.

The thyroid can be removed in the adult animal without the production of any changes. In July, 1914, the thyroids were removed from four spermophiles. These hibernated normally during the winter of 1914–15 and two were alive and hibernated during the fall of 1915. During the summer of 1915 the thyroids were removed from twelve animals. These hibernated under the same condition as the controls. In the animals operated on histologic examination showed that the thyroid was removed although it was impossible to state that all thyroid tissue had been taken out. Necropsies were performed on practically all the animals and in only one was any thyroid tissue found on macroscopic examination.

The parathyroids. The parathyroids are relatively large in the spermophile. They are found not only attached to the thyroids but also buried in the hibernating gland in the neck or anterior mediastinum. The histologic examination of these glands shows that in some animals they undergo very slight changes during hibernation. These changes consist in a decrease in their ability to take up the stains and a more irregular arrangement of the cells. In many hibernating animals they cannot be observed.

The thymus. A considerable amount of lymphoid tissue is always found buried in the substance of the hibernating gland in the anterior mediastinum. Hassall's corpuscles were noted in only a few of the animals examined. In some cases the staining ability of the lymphoid tissues and corpuscles of the gland from the hibernating animal seemed to be decreased. This decrease, however, was not uniform and so slight as not to warrant any positive statement.

The islands of Langerhans. The histologic picture of the pancreas of a hibernating spermophile does not differ essentially from that of a resting pancreas. The islands of Langerhans are large and well developed in the spermophile, and the A and B cells are well differentiated. The only changes noted in the islands of the hibernating animal were a very slight decrease in staining properties and an irregularity in the arrangement of the cells. This change is not uniform.

The adrenals. The adrenal of the spermophile is a small but perfect gland having a medulla and cortex with its three fairly well de-

fined zones. This gland undergoes fairly definite and uniform seasonal changes. However, these changes have not been observed in all animals. The adrenal shows a gross increase in size in the spring and on histologic examination all the cells of both the cortex and medulla are found to be swollen. The main change takes place in a stratum of cells which are located in the inner part of the columns of the zona fasciculata. These cells are greatly swollen and very large in the spring. As the season advances the size of the adrenal decreases. Histologically all the cells appear to become smaller but the major changes consist in a marked decrease in the cells of the inner portion of the zona fasciculata and a vacuolization of the cells of the zona granulosa and the upper portion of the zona fasciculata. During hibernation the latter changes seem to be accentuated but the lipoid content does not seem to change greatly. The medullary cells shrink very slightly during hibernation.

I was surprised to discover that the adrenals could be removed from the spermophile without producing immediate death, except in a small percentage of cases. In a few instances the animals died typical deaths from adrenal insufficiency. As a rule the animal recovered from the operation and either became perfectly normal or more frequently lost weight and strength gradually, dying several weeks after removal of the last gland. In some cases necropsy revealed a hypertrophied accessory adrenal but in most cases a diligent search with the bichromate method and histologic examination of suspicious particles did not reveal any adrenal tissue. (4) This probably does not mean that the spermophile can live without adrenal tissue or that another gland vicariously takes up its function, but that scattered bits of adrenal tissue which were too small to be found without serial section of the whole animal were enough to keep the animals alive for various lengths of time. However, it is of particular importance in the study that, at any event, the animal in which the adrenals were removed had a minimum amount of functionating adrenal tissue. This was amply proved by the fact that most of the animals became thin and weak and died.

The adrenals were removed from a series of animals in July, 1914. Five of them lived and hibernated during the winter of 1914–15. Twelve additional adrenalectomized animals were studied during the fall of 1915 and were found to hibernate under the same condition as the controls.

While these results show that the adrenals undergo fairly constant seasonal changes, they are just as much indicative of increased functional activity during the period of rut as of decreased activity during hibernation. It would appear that the adrenals do not bear any causal relation to hibernation for two reasons. First, the adrenalectomized animals, which certainly had only a minimum amount of adrenal tissue, hibernated normally. Second, the histologic changes noted in the hibernating animals were progressive, being found to some extent in the active animals in the autumn, and quite as marked in the animals which were sacrified during the winter and which had not hibernated owing to the high temperature of the room.

The pituitary. The spermophile has a well-formed pituitary gland. It does not differ from the corresponding glands of other species except that the differential staining properties of the cells of the anterior glandular portion are not very marked. I have not been able to note any seasonal variation in the pituitary. In some of the pituitary glands of hibernating spermophiles I have found the same changes observed by Cushing and Goetsch in the woodchuck. These consist in an almost complete loss of the normally rather slight differential staining qualities of the granular content of the cells to acid and basic dyes, a decrease in the size of the cells and an irregularity in the cellular grouping. However, these changes are not constant. In several hibernating spermophiles I have not been able to distinguish any changes in the pituitary glands. In many, the changes are very slight. No mitotic figures were seen.

The inconstancy of the changes is proved very easily by a study of the glands having controls. I am not able to see any difference between the pituitaries of Gophers 225 and 227; 114 and 270, or 271 and 272.

The results of the study were as follows:

1. The sex glands undergo a definite seasonal variation but certainly do not play any part in the cause of the hibernating state or undergo any specific changes due to the torpid condition.

2. I have been unable to determine any changes in the thyroids of the hibernating animal, and the fact that animals in which the thyroids were removed hibernated normally tends to show that these glands are not factors of significance in hibernation.

3. The very slight and inconstant changes noted in the parathyroids do not warrant any positive conclusion.

4. The thymus does not appear to undergo any uniform change.

5. The islands of Langerhans undergo changes so slight as not to justify a positive conclusion.

6. The adrenal glands undergo a fairly definite seasonal variation. The different changes are most marked during the winter and during the period of rut. However, the adrenals do not appear to be a specific factor in hibernation because the same changes were noted in animals sacrificed during the winter which had not been allowed to hibernate, and removal of the adrenals, which certainly reduces the adrenal substance to a minimum, did not in any way change the hibernating ability of the animal.

7. Some of the pituitary glands of the hibernating animals showed definite changes, but these changes were not constant.

CONCLUSION

It is impossible to state that some of the ductless glands are not of importance in relation to hibernation. New methods of investigation may demonstrate this relationship. However, the results of this study do not justify the assumption of any theory ascribing the phenomena to a lack of function of all or any one of the ductless glands.

BRIEF RECORDS OF MOST OF THE SPERMOPHILES WHOSE DUCTLESS GLANDS WERE STUDIED

A. Active animals

No.

105 Female; weight 100 grams; captured April, 1915; pregnant; fasted for five days. Sacrificed May 5, 1915.

106 Male; weight 135 grams; captured April, 1915; fasted for five days. Sacrificed May 5, 1915.

108 Female; captured with two of her young in June, 1914. Very savage. Was kept in warm room during the winter of 1914–15, but hibernated when the temperature was allowed to decrease below 20°C. Sacrificed May 21, 1915.

147 Female; weight 125 grams; captured June, 1915. Sacrificed June 12, 1915.

148 Female; captured June, 1915. Sacrificed June 12, 1915.

149 Male; weight 143 grams; captured June, 1915. Sacrificed July 20, 1915.

174 Female; weight 144 grams; captured June, 1915. Sacrificed July 20, 1915.

175 Male; weight 134 grams; captured June, 1915. Sacrificed July 20, 1915.

192 Female; captured July, 1915. Had lactated. Sacrificed August 3, 1915.

198a Female; captured in the spring of 1915. Sacrificed August 14, 1915.

199a Female; captured in the spring of 1915. Sacrificed August 14, 1915.

204 Male; captured season 1915. Sacrificed September 14, 1915.

240 Female; very fat; weight 180 grams; captured early in the spring of 1915. Sacrificed September 24, 1915.

No.

241 Male; captured in the spring of 1915. Sacrificed September 24, 1915.

246 Female; captured in the spring of 1914. Both ovaries removed July 10, 1914. During the winter of 1914–15 was allowed to hibernate for short periods, but was active most of the time. Sacrificed September 21, 1915. Weight at time of death, 95 grams.

142 Female; weight 135 grams; captured May, 1915. Both ovaries removed June 3, 1915. Animal became slightly torpid at times during the autumn months, but was kept in a very warm room and did not hibernate. Sacrificed November 10, 1915.

146 Female; weight 128 grams; captured May, 1915. Uterus removed June 11, 1915. Sacrificed July 19, 1915. Weight at this time, 135 grams.

251 Female; weight 101 grams; captured July, 1914. Uterus and left ovary removed October 5, 1915. Sacrificed October 26, 1915.

225 Male; weight 110 grams; born in the spring of 1915. Animal placed in hibernating room without food at 12.00 m., November 11, 1915. Remained very active until November 20, when it seemed slightly torpid. Rectal temperature 20°C. Sacrificed at this time. (Control for Spermophile 227.)

114 Female; weight 120 grams; captured June, 1915. Animal was well supplied with food and kept in warm room. Did not become torpid while in captivity. Sacrificed February 2, 1916. (Control for Spermophile 270).

268 Female; weight 55 grams; born in the spring of 1915. Animal had never hibernated. Food withdrawn but room kept warm, February 3, 1916. Sacrificed February 5, 1916. (Control for Spermophile 267.)

269 Female; weight 50 grams; born in the spring of 1915. Animal had never hibernated. Sacrificed February 24, 1916. (Control for Spermophile 220.)

271 Female; weight 140 grams; captured July, 1915 Had lactated that season. Did not hibernate while in captivity. Sacrificed February 28, 1916. (Control for Spermophile 272.)

275 Male; weight 105 grams; captured in the spring of 1915. Spleen removed July, 1915. Was not allowed to become torpid while in the laboratory. Sacrificed March 27, 1916. Genital tract showed the marked hypertrophy and hyperemia of beginning rut. (Control for Spermophile 274.)

278 Female; weight 85 grams; captured July, 1915. Had lactated in the spring of 1915. Did not hibernate during the winter of 1915–16. Sacrificed April 11, 1916. At necropsy the animal showed pregnancy of a few days' duration.

284 Female; weight 65 grams; captured July, 1915. Born in the spring of 1915. Did not hibernate during the winter of 1915–16. Sacrificed April 21, 1916.

B. Hibernating animals

Time hibernating	
267 12 hours	Female; weight 60 grams; born in the spring of 1915; kept under observation and it is certain that it had never become torpid until placed in the hibernating room without food February 4, 1916, at 4.00 p.m. It became torpid about 10.00 p.m. Was sacrificed February 5, at 10.00 a.m. Rectal temperature 11°C.

No.	*Time hibernating*	
252	1 day	Fat female; captured in the spring of 1915. November 8, 1915 at 4.00 p.m. placed in room with temperature of 14° C. with no food. Had not hibernated previously during the season. Remained active until 10.00 am., November 9. Sacrificed November 10 at 10.00 a.m. Rectal temperature 14°C.
253	2 days	Fat female; captured in the spring of 1915. November 8, 1915, placed in room to hibernate, with no food. Did not become torpid for several hours. Sacrificed November 12, after hibernating for two days. Temperature of room at this time 9°C. Rectal temperature 11°C.
255	3 days	Fat female; captured in the spring of 1915. Placed in hibernating room, without food. Remained active for several hours. Sacrificed November 18, 1915, after hibernating three days. Rectal temperature 12°C.
127	4 days	Male; weight 150 grams; captured in the spring of 1915. Placed in hibernating room without food; remained there less than twenty-four hours. Sacrificed December 2, 1915, after being torpid four days. Rectal temperature 14°C. Room temperature 12°C.
227	5 days	Male; weight 108 grams; born in the spring of 1915. Animal placed in hibernating room, without food, at 12.00 m., November 11, 1915. Became torpid first November 16. Sacrificed November 20, at 10.30 a.m. Rectal temperature 10°C.
254	6 days	Very fat female; captured in the spring of 1915. When placed in hibernating room without food, became torpid very quickly. Sacrificed November 15, 1915, after hibernating six days. Rectal temperature 8.5°C. Room temperature for the six days varied from 5° to 10°C.
226	7 days	Male; weight 96 grams; born in the spring of 1915. September 9, 1915, both testes removed. Animal recovered from operation and increased in weight. Placed in hibernating room without food; sacrificed December 15, 1915, after hibernating seven days. Rectal temperature 9.5°C. Weight when sacrificed 110 grams.
132	8 days	Female; weight 115 grams. Captured May, 1915. Placed in hibernating room without food; sacrificed December 11, 1915, after being torpid eight days. Rectal temperature 15°C.
259	9 days	Female; weight 100 grams; captured in the spring of 1915. Placed in hibernating room without food; sacrificed December 17, 1915, after hibernating nine days. Room temperature at this time 0°C. Rectal temperature 5°C.
256	10 days	Very fat female; captured in the spring of 1915. Placed in hibernating room without food; sacrificed November 20, 1915, after hibernating ten days. Rectal temperature 11.5°C.
260	15 days	Female; weight 85 grams; captured in the spring of 1915. Animal had been torpid continuously for fifteen days, although it had been torpid for a short time earlier in the season. Did not have access to food; sacrificed January 1, 1916. Rectal temperature 12°C.

No.	Time hibernating	
170	20 days	Female; weight 145 grams; captured in the spring of 1915. Animal did not have access to food; sacrificed January 15, 1916, after being torpid approximately twenty days. Rectal temperature 14°C.
258	25 days	Male; weight 150 grams; captured in the spring of 1915. Did not have access to food; sacrificed December 11, 1915, after hibernating twenty-five days. Rectal temperature 12.5°C.
257	30 days	Very fat female; weight 195 grams; captured in the summer of 1915. Had lactated that season. Did not have access to food; sacrificed December 11, 1915, after being torpid thirty days. Rectal temperature 12.5°C.
117	35 days	Male; weight 165 grams; captured in the spring of 1915. Animal had access to food. Had been torpid thirty-five days. Sacrificed January 10, 1916. Rectal temperature 14°C.
261	40 days	Very fat male; weight 150 grams; captured in the spring of 1915. Had access to food. Sacrificed January 12, 1916, after hibernating forty days. Rectal temperature 2.5°C.
221	45 days	Female; weight 85 grams; born in the spring of 1915. Both thyroids removed September 20, 1915. Placed in hibernating room without food; sacrificed January 13, 1916, after being torpid continuously for forty-five days. Rectal temperature 6°C.
121	50 days	Male; weight 180 grams; captured May, 1915. Sacrificed January 13, 1916. It had been hibernating for fifty days without access to food. Had shown some signs of activity for a short time during this period but did not become active. Rectal temperature 5°C.
263	75 days	Female; weight 100 grams; captured in the spring of 1915. Sacrificed January 12, 1916, after hibernating seventy-five days without access to food. This animal was slightly active a few times during this period. Rectal temperature 12°C.
272	80 days	Female; weight 90 grams; captured in the spring of 1915. Sacrificed February 28, 1916. Had been hibernating without access to food for eighty days. Rectal temperature 19°C. Daily observations were not taken on this animal.
262	100 days	Male; weight 95 grams; captured in the spring of 1914. Hibernated at intervals during the winter of 1914–15. Food was withdrawn October 1, 1915, and the room allowed to approximate outside temperature. Animal immediately became torpid. Sacrificed January 12, 1916, after hibernating about one hundred days. Animal rather thin. Rectal temperature 11°C. Daily notes not taken.
266	125 days	Female; captured June, 1914. Hibernated when kept in cold during the winter of 1914–15. Placed in hibernating room and food withdrawn the latter part of September, 1915. Sacrificed January 27, 1916, after hibernating about one hundred and twenty-five days. Rectal temperature 17°C. Daily observations were not made.

No.	Time hibernating	
270	150 days	Female; captured May, 1914. Hibernated at intervals during the winter of 1914–15. Was placed in hibernating cage, without food, October 1, 1915. Daily observations were not taken, but it is known that the animal was torpid most of the time, although at intervals it was slightly warm to the touch. Sacrificed February 28, 1916. Quite torpid; rectal temperature 21°C. Very thin. Weight 75 grams.
220	Indefinite	Male; weight 93 grams; born in the spring of 1915. Both thyroids removed September 20, 1915. Hibernated at intervals throughout the winter. Had access to food. Sacrificed February 24, 1916. Quite torpid. Rectal temperature 20°C. Very thin; weight 50 grams. No thyroid tissue found. Daily observations not taken.
228	Indefinite	Male; weight 82 grams; born in the spring of 1915; both testicles removed September 21, 1915. Hibernated at intervals throughout winter. Had access to food. Sacrificed February 28, 1916. Quite torpid. Rectal temperature 20°C. Weight 65 grams. Daily observations not taken.
135	Indefinite About 40 days	Female; weight 122 grams; captured May, 1915. Spleen removed May 28, 1915. Animal recovered from operation and became fat. December 13, 1915, placed in hibernating room with food. In the attempt to determine the reaction of the hibernating animal to excessive cold, it froze to death January 13, 1916. Tissues of all organs were well preserved.
118	Indefinite About 45 days	Female; weight 105 grams; captured in the spring of 1915. Placed in hibernating room with food December 3, 1915. In studying effect of excessive cold upon hibernating animals, it was frozen to death January 13, 1916. Tissues were in excellent preservation.
264	Indefinite About 40 days	Female; weight 95 grams; captured in the spring of 1915. Placed in hibernating room with food December 3, 1915. Was frozen to death during the study of the effect of excessive cold upon hibernation, January 13, 1916. Tissues well preserved.
265	Indefinite About 40 days	Male; weight 95 grams; captured in the spring of 1915. Placed in hibernating room with food December 3, 1915. Was frozen to death during the attempt to determine the effect of excessive cold upon hibernation, January 13, 1916. Tissue well preserved.

C. Active immediately after hibernation

237	Male; born in the spring of 1915; right adrenal removed September 23, 1915. Had access to food throughout winter. Hibernated at intervals. Sacrificed March 11, 1916. Animal very active at this time. Very poor weight—50 grams.
210	Female; captured in the spring of 1915; right adrenal removed September 15, 1915. Had access to food throughout the winter. Hibernated at rare intervals. Sacrificed March 11, 1916. Animal very fat; weight 110 grams.

No.

126 Female; captured in the spring of 1915. December 12, 1915, placed in his bernating room without food. While daily observations were not made, it is known that the animal was very torpid although two or three times it was found to be active. At 9.00 a.m., March 11, 1916, animal was torpid; rectal temperature 15°C. Animal was placed in warm room and at 10.00 a.m. was very active. Sacrificed at 3.00 p.m. Weight 75 grams.

273 Female; weight 95 grams. Captured late in the spring of 1915. Had lactated during the season. Hibernated at intervals during the winter of 1915–16. Had access to food. Was hibernating March 11, when it was placed in a warm room, and remained active. Sacrificed March 18.

231 Female; weight 200 grams. Captured in the spring of 1915. Ovaries removed September 22, 1915. Hibernated at intervals throughout winter 1915–16. Had access to food. Sacrificed March 18, 1915; weight at this time 100 grams.

274 Male; weight 85 grams. Captured in the spring of 1915. Hibernated during the winter of 1915–16. Had access to food. Was torpid until March 11, when its rectal temperature was 20°C. When placed in a warm room, it remained active. Sacrificed March 27.

276 Female; weight 105 grams. Captured in the spring of 1915; hibernated during the winter of 1915–16. Had access to food. Active after March 11. Sacrificed March 27.

190 Female; weight 106 grams. Captured in the spring of 1915. July 23, 1915, complete removal of both thyroids. Animal hibernated during the winter of 1915–16. Had access to food. It was placed in a warm room March 11, and thereafter was never noted to be torpid. Sacrificed April 4; weight at this time 83 grams.

273 Male; weight 145 grams. In excellent condition. The first animal caught in the spring of 1916. Captured and sacrificed April 11, 1916. Marked hyperemia and hypertrophy.

280 Female; weight 120 grams. Captured July, 1915. Had lactated during the season of 1915. Hibernated during the winter of 1915–16. Had access to food. Was not torpid after March 10. Sacrificed April 14, 1916.

281 Female; weight 145 grams. Captured and sacrificed April 14, 1916.

282 Male; weight 170 grams. Captured and sacrificed April 14, 1916.

283 Female; weight 110 grams. Captured and sacrificed April 17, 1916.

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(1) Cushing and Goetsch: Hibernation and the pituitary body, Jour. Exp. Med., 1915, xxii, 25.

(2) Polimanti: Il letargo, 1913. This is the most recent book dealing with the subject. It is very thorough and gives a very full bibliography.

(3) Gemelli: Su l'ipofisi delle marmotte durante il lethargo e nella stagione estiva, Arch. per le scienze mediche, 1906, xc, 941.

(4) Vincent in Internal Secretion and the Ductless Glands, 1913, 116, states that he has never been able to find chromophile bodies in the spermophile.

THE REGULATION OF THE BLOOD VOLUME AFTER INJECTIONS OF SALINE SOLUTIONS[1]

Studies of the Permeability of Cellular Membranes. I

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Received for publication, June 14, 1916

The volume of the blood is one of the important constants of the body, the maintenance of which constitutes a condition of health. With widely varying fluid intake, there must frequently be a tendency toward disturbance of this equilibrium and toward an alteration in the volume of the blood. The mechanism by means of which a constant blood volume is normally maintained is, therefore, of very general physiological interest.

Attention has again been directed to these subjects by recent investigations. Boycott ('13) found that, in normal rabbits, the original volume of the blood was restored in a short and fairly constant interval after intravenous injection of Ringer's solution. On the other hand, rabbits rendered nephritic by uranium nitrate showed a partial failure of the blood volume to return to normal under the same experimental procedure. Chisholm ('14 a) found a similar incomplete regulation of the blood volume in rabbits with nephritis induced by potassium chromate; and likewise ('14 b) in normal rabbits after the injection of extract of kidneys from animals with uranium or chromate nephritis.

The present series of investigations, suggested by the work of Boycott and Chisholm, was undertaken with the object (1) of studying further the phenomena described by them in normal and nephritic rabbits; (2) of determining whether a similar disturbance of the regulation of the blood volume was to be found in rabbits suffering from tartrate nephritis; and (3) of extending these studies to the dog, in

[1] The data in this paper are taken from the dissertation presented by L. Jean Bogert for the degree of Doctor of Philosophy, Yale University, 1916.

order to follow the flow of lymph from the thoracic duct in normal and nephritic animals after injections into the blood stream.

The general plan of experimentation employed in the work of Boycott ('13 and '14) and Chisholm ('14, a and b) has been followed in the present investigation. After the concentration of hemoglobin in the blood was determined, a known quantity of fluid was forced into the circulation under pressure as rapidly as possible. Immediately after the injection and at regular intervals thereafter the concentration of hemoglobin was measured and the relative blood volumes were calculated from these figures.

Technique

Ringer's solution,[2] warmed to 38°C., was used for the injections of saline solution. The fluid was usually introduced into the ear vein in rabbits and into the femoral vein in dogs. The injections were made under pressure and generally at the rate of about 50 cc. per minute. Samples of blood were taken at first from the vessels of the rabbits' ear but it was found that such samples did not give constant results so that all blood samples were later taken from the carotid artery in rabbits. In the case of dogs, the blood samples were uniformly taken from the femoral artery. Only a few drops of blood were required for hemoglobin determination. These were caught in depressions in a paraffin plate and determinations made at once to avoid loss of water by evaporation. Rabbits were anaesthetized with ether only, but dogs generally received a subcutaneous injection of 10 mg. of morphine sulphate and one of atropine sulphate per kilogram of body weight one hour previous to anaesthetization with ether. Hemoglobin determinations were made according to the method suggested by Haldane,[3] the accuracy of the standard used having been checked by determinations of the oxygen content of blood, made by the Haldane-Barcroft method.

Calculation of the original blood volume of rabbits was made on the assumption (Boycott '13) that they have about 50 cc. of blood per kilogram of body weight. They usually received injections of this amount of fluid; but the dogs only half as much or 25 cc. per kilogram of body weight. In some cases the animal was given a second or third injection. An illustrative example of the method of calculating the relative blood volumes follows: if the original blood volume is taken as 100 per cent and if the content of hemoglobin was 60 per cent before injection and 40 per cent after injection, the relative blood volume after injection would be $\frac{60}{40} \times 100 = 150$ per cent.

The accuracy of such calculations depends, obviously, on the assumption that the amount of hemoglobin in the circulation is constant. Experimental data

[2] This solution contained 0.9 per cent sodium chloride, 0.024 per cent calcium chloride, 0.042 per cent potassium chloride and 0.03 sodium bicarbonate.

[3] Haldane: Journ. of Physiol., 1901, xxvi, 497.

have been published recently to show that the number of red blood corpuscles may be increased in high altitudes[4] or when work,[5] causing an appreciable amount of perspiration, is done. Lamson[6] also found increased red corpuscle content of the blood as a result of partial asphyxiation or fright. Increase in the number of red blood corpuscles may, of course, take place without an increase of the amount of hemoglobin in the circulation; but, in order to test the validity of the method used, a number of control experiments were made.

CONTROL EXPERIMENTS

Hemoglobin content in the same animal at intervals. The concentration of hemoglobin in the blood was determined at half hour intervals in four normal rabbits and the results are given in the table below.

TABLE 1

Hemoglobin content in the same animal at intervals

	HEMOGLOBIN IN PER CENT							
	0 hr.	½ hr.	1 hr.	1½ hr.	2 hr.	2½ hr.	3 hr.	3½ hr.
Rabbit A	54	53	54	53				
Rabbit B	70	69	70					
Rabbit C	68	68	69	68	68	69	68	69
Rabbit D	73	74	74	73	74			

Comparison of hemoglobin and total solid content of the blood after injections of saline solution. The ordinary injection of 25 cc. of Ringer's solution per kilogran of body weight was given to a number of dogs, samples of blood being taken before and after the injection and at intervals later. The percentage of hemoglobin in the blood was determined and, in addition, the percentage of water was also ascertained by drying to constant weight at 100 to 110°C. The results of these experiments are given in the following table:

[4] Schneider and Havens: This Journal, 1915, xxxvi, 380.
[5] Boothby and Berry: This Journal, 1915, xxxvii, 378.
[6] Lamson: Journ. of Pharm. and Exper. Therap., 1915, vii, 169.

TABLE 2

Comparison of the hemoglobin and total solid content of the blood after injections of saline solutions

TIME	EXPERIMENT 43		EXPERIMENT 60		EXPERIMENT 61		EXPERIMENT 65	
	Hemoglobin	Total solids	Hemoglobin	Total solids	Hemoglobin	Total solids	Hemoglobin	Total solids
	per cent		*per cent*		*per cent*		*per cent*	
Before injection	44	13.6	77	20.7	83	22.8	70	21.9
After injection								
Immediately	31	10.7	55	16.0	66	18.5	55	17.6
10 minutes					74		60	
20 minutes					78		63	
25 minutes	40	12.7						
30 minutes			70	20.5	83	22.9	63	20.5
40 minutes	40	13.1			84		64	
50 minutes					83		64	
60 minutes			76	21.3	84	22.7	64	20.4

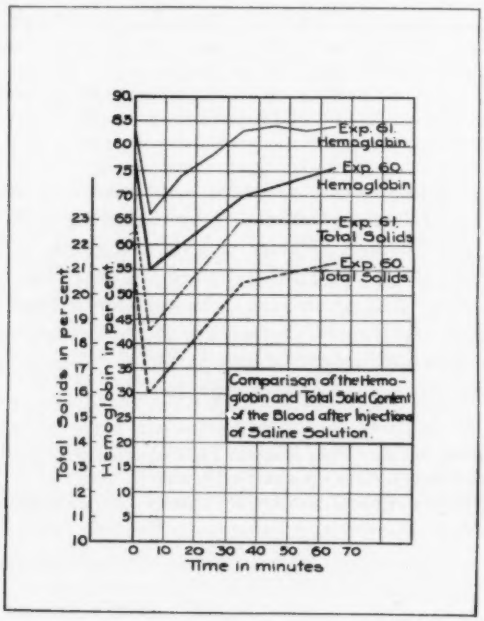
Exp. 61 Hemoglobin
Exp. 60 Hemoglobin
Exp. 61 Total Solids
Exp. 60 Total Solids
Comparison of the Hemoglobin and Total Solid Content of the Blood after Injections of Saline Solution.
Total Solids in per cent.
Hemoglobin in per cent.
Time in minutes
0 10 20 30 40 50 60 70

Fig. 1

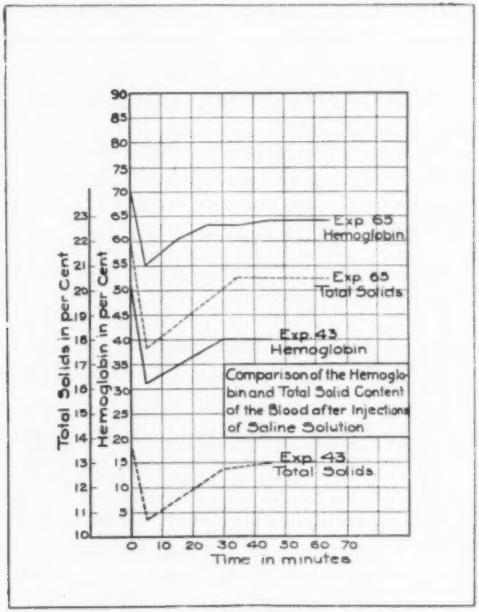
Total Solids in per Cent
Hemoglobin in per Cent
Time in minutes
Exp. 65 Hemoglobin
Exp. 65 Total Solids
Exp. 43 Hemoglobin
Exp. 43 Total Solids
Comparison of the Hemoglobin and Total Solid Content of the Blood after Injections of Saline Solution

Fig. 2

The first set of experiments (table 1) clearly shows that the hemoglobin does not vary in amount in individual animals, at least during periods of several hours duration. The second series of experiments (table 2) establishes the fact that the hemoglobin is a true measure of the dilution of the blood, since the results obtained by this method are in close accord with those obtained by the determination of the total solid content of the blood. When the figures in table 2 are represented graphically (see figs. 1 and 2), the parallelism of the hemoglobin and total solid curves is seen to be almost perfect. Under the conditions of these experiments, then, it is safe to conclude that the amount of hemoglobin in the circulation does not alter materially and, therefore, it can be used as a measure of the dilution of the blood.

EXPERIMENTS WITH RABBITS

Under normal conditions. A specimen protocol is given below. The results of thirteen such experiments are summarized in table 3.

Experiment 21. Rabbit ♀, normal, weighing 2140 grams. Injection of 100 cc. of Ringer's solution into the ear vein in 2 minutes; blood samples from carotid artery.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	37	100
After injection		
Immediately	23	160
5 minutes	32	116
10 minutes	35	106
15 minutes	37	100
20 minutes	37	100

No fluid in pleural cavity; about 3 cc. in peritoneal cavity.

TABLE 3

Regulation of the blood volumes in normal rabbits after injections of saline solution

EXPERIMENT NUMBER	RELATIVE BLOOD VOLUMES AFTER						
	5 min.	10 min.	15 min.	20 min.	25 min.	30 min.	35 min.
1		131	128	109	100	100	100
2		121	112	105	100	101	100
3			115	104	98	100	100
4	150	133	113	107	101	100	100
5	136	114	116	105	103	100	
6	152	139	134	131	117	113	101
7	141	120	114	109	107	101	100
11	151	127	118	106	103	104	
12	143	121	114	105	100	100	
13	137	114	106	102	100	100	
21	160	116	106	100	100		
22	130	114	107	104			
23	159	130	123	110	105		
Average..	146	121	116	107	103	102	100

From these figures it appears that a great deal of the fluid introduced leaves the blood stream during the period of the injection. The more rapid the introduction of the solution the less is the amount of liquid able to leave the circulation during this period. In experiment 3, where the duration of the injection was fifteen minutes, the blood volume was found to be only 15 per cent above normal at the close of the injection, while the greatest increase in relative blood volume noted

was in experiment 21, where the solution was introduced in two minutes. It is, however, to be remembered that the calculated original blood volume is only approximate and that undoubtedly some animals received more fluid than others, in proportion to their original blood volume.

In spite of some individual variations, the figures show a satisfactory agreement. The initial dilution caused by the injection varies somewhat, due largely to the causes outlined in the preceding paragraph; but the length of time required for the blood volume to return to normal is about twenty-five minutes in almost every case. It will be observed that the fluid leaves the blood stream most rapidly in the earlier moments after the injection; i.e., the stimulus to rid the blood of the excess of fluid is strongest when the excess is greatest.

In general, then, it was found that, after injection of a quantity of saline solution equal to the calculated blood volume, into normal rabbits, the excess of fluid disappears from the circulation rapidly and the original blood volume is restored in a short time, usually not more than thirty minutes.

After ligation of the kidneys. In order to determine the influence of the kidney factor in the regulation of the blood volume in normal rabbits, the kidneys were ligated at the hilum and injections of Ringer's solution were then made. In some of these experiments the blood volume returned to normal in the same length of time as in normal rabbits with the kidneys active. Others showed a very slight delay. Of the two protocols given here, that of experiment 19 is an example of the former class and experiment 17 of the latter.

Experiment 19. Rabbit ♀, both kidneys ligated, weighing 1780 grams. Injection into ear vein of 100 cc. of Ringer's solution in 2 minutes; blood samples from carotid artery.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	40	100
After injection		
Immediately	28	142
5 minutes	34	118
10 minutes	36	111
15 minutes	38	105
20 minutes	40	100
25 minutes	40	100
30 minutes	39	102
45 minutes	39	102
55 minutes	40	100

Killed one hour after injection. Kidneys found completely ligated; no fluid in pleural or peritoneal cavities.

Experiment 17. Rabbit ♀, both kidneys ligated, weighing 2200 grams. Injection into ear vein of 100 cc. of Ringer's solution in 2 minutes; blood samples from carotid artery.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	55	100
After injection		
Immediately	39	141
5 minutes	48	114
10 minutes	50	110
15 minutes	51	108
20 minutes	52	106
25 minutes	53	104
30 minutes	54	102
35 minutes	53	104
50 minutes	54	102
60 minutes	55	100

Killed one hour after injection. Kidneys found completely ligated; no fluid in pleural and peritoneal cavities.

From these experiments it seems that ligation of the kidneys produces no marked effect on the regulation of the blood volume. Boycott ('13 and '14) and Chisholm ('14 a and b), ligated the ureters just above the bladder in all their normal animals and their results are very similar to those of the experiments recorded in table 3, in which healthy rabbits with active kidneys were used. Moreover, in experiments 1 to 13, the urine was collected by compression of the bladder at half hour or hour intervals; and there was no connection whatever between the amount of urine secreted and the rate of restoration of the blood volume to normal. The amount of urine secreted varied widely. In some animals a volume of urine equal to or greater than the volume of the injected fluid, was secreted in four or five hours, while in others only about one-fourth of the injected fluid had been excreted after three or four hours. In this connection, it is interesting to note that some of the rabbits which showed a poor excretion of the fluid by the kidneys had diarrhoea the following day. At the end of thirty minutes, however, when the blood volume had in practically every case returned to normal, only 5 to 28 per cent of the injected volume had left the

body as urine. Autopsy showed no fluid in the body cavities. It seems clear, therefore, that the tissues act as a reservoir for the excess of fluid, which later leaves the body gradually, and that the activity of the kidney plays only a small rôle in the regulation of the blood volume after intravenous injection of salt solutions.

After starvation and lack of water. To ascertain whether the previous condition of the tissues had any effect on the regulation of the blood volume, three rabbits were kept without food or water for three days before injection. These experiments were also designed as controls for the experiments with nephritic rabbits, which frequently refuse food and water.

Experiment 10. Rabbit ♀, weighing 1660 grams, deprived of food and water for three days. Injection of 100 cc. of Ringer's solution in 2 minutes.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME	URINE
	per cent		*cc.*
Before injection	56	100	
After injection			
Immediately	44	127	
5 minutes	46	123	
10 minutes	51	110	
15 minutes	54	104	
20 minutes	56	100	
25 minutes			8
1 hour			3
1½ hours			2
2 hours			5
2½ hours			12
3 hours			13
			43

TABLE 4

Regulation of blood volume after starvation and lack of water

EXPERIMENT NUMBER	RELATIVE BLOOD VOLUMES AFTER					
	5 min.	10 min.	15 min.	20 min.	25 min.	30 min.
8	188	138	119	111	106	
9	123	119	109	106	103	100
10	127	123	110	104	100	

The figures in the above table indicate that a condition of food and water deprivation has little or no influence on the rate with which in-

jected fluids leave the blood stream. Probably the capacity of the tissues to act as a reservoir for excess of fluid is so great that the quantities of liquid dealt with in these experiments have no appreciable effect on the mechanism for maintaining constant blood volume. In experiments 9 and 10 there seems some support for the idea that tissues poor in water draw the excess of fluid from the blood stream more rapidly, since the relative blood volumes immediately after injection are considerably below the average figure for normal rabbits (see table 3). In experiment 8, however, the initial dilution is unusually high, which cannot be accounted for by the rapidity of the injection, as the rate of injection was the same in all three experiments.

In uranium nephritis. The rabbits were rendered nephritic by subcutaneous injection of 10 mg. of uranium nitrate per kilogram of body weight, the same dosage as that used by Boycott ('13 and '14). Although the characteristic incomplete recovery of the normal blood volume after injections into the blood stream is usually apparent on the second day after the injection of the uranium nitrate, it may be absent, in case the nephritis is not sufficiently advanced; and it is much more marked on the fourth or fifth day. As anuria sets in about the third day of the nephritis and death frequently results by the fifth day, the fourth day after administration of the uranium nitrate was found to be the best time for experimental purposes.

Experiment 36.[7] Rabbit ♀, nephritic, weighing 2000 grams.

July 19—Subcutaneous injection of 10 mg. of *uranium nitrate* per kilogram.

July 22—About 6 cc. of urine which gave tests for protein and sugar. Animal was in poor condition, lethargic and breathing irregularly.

Injection of 100 cc. of Ringer's solution in 1 minute, 40 seconds.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	65	100
After injection		
Immediately	35	186
5 minutes	43	151
10 minutes	49	133
15 minutes	53	123
20 minutes	51	127
25 minutes	54	120
30 minutes	53	123

[7] These and the following protocols have been selected as examples from a large number of duplicate experiments.

Injection II of 100 cc. of Ringer's solution in 1 minute, 45 seconds.

After injection		
Immediately	34	191
5 minutes	40	163

Rabbit died suddenly, with foaming at mouth and nose, ejection of a colorless watery fluid and convulsive struggles. Kidneys were very pale, with no edema or hypertrophy. The bladder contained 8 cc. of urine, the peritoneal cavity contained no fluid but there were 10 cc. of clear fluid in the pleural cavity.

Experiment 35. Rabbit ♀, nephritic, weighing 2200 grams.

July 19—Subcutaneous injection of 10 mg. *uranium nitrate* per kilogram.

July 21—100 cc. of urine which gave a flocculent precipitate on heating and reduced Benedict's solution.

Injection of 110 cc. of Ringer's solution in 1 minute 45 seconds.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	62	100
After injection		
Immediately	38	163
5 minutes	49	126
10 minutes	53	117
15 minutes	53	117
20 minutes	55	113
25 minutes	55	113
30 minutes	55	113
35 minutes	55	113

Injection II of 110 cc. of Ringer's solution in 2 minutes.

After injection		
Immediately	34	182
5 minutes	46	135
10 minutes	50	124
15 minutes	50	124
20 minutes	53	117
25 minutes	53	117
30 minutes	55	113

Kidneys were pale, with no edema and the bladder was empty. There was no fluid in the pleural cavity, while the peritoneal cavity contained 20 cc. of clear fluid which clotted rapidly.

Experiment 36 shows a distinct disturbance of the mechanism for regulation of the blood volume associated with the nephritic condition. Not only is the initial dilution caused by the injection greater than in normal rabbits, indicating the inability of the excess of fluid to leave the circulation as rapidly, but at the end of thirty minutes when the injected fluid would have completely disappeared from the blood stream of a normal rabbit, 23 per cent of it is still present in the blood stream of the nephritic animal. Moreover, all the fluid removal took place during the first fifteen minutes after the injection. During the last fifteen minutes of the experiment, it had apparently been impossible for the organism to remove any more liquid from the circulation. An attempt was made to determine the effect of a second injection but the animal died.

The effects noted under experiment 36 are reproduced in experiment 35, although they are not so pronounced. This is probably due to the fact that the animal employed had been subjected to the influence of uranium nitrate for a period of two days only. A second injection was here successful and was followed by increased initial dilution of the blood and a slower rate of removal of the excess of fluid. It is interesting to note that the same extent of recovery of the original blood volume (113 per cent relative blood volume) took place after each injection and that further removal of fluid seemed to be impossible.

In tartrate nephritis. Since Chisholm ('14 a) had found the same disturbance of the regulation of the blood volume in rabbits with chromate nephritis, it seemed of interest to ascertain whether a similar condition prevailed in rabbits suffering from a nephritis induced by sodium tartrate. The procedure suggested by Underhill, Wells and Goldschmidt[8] was followed. The rabbits were given no food for two days prior to receiving a subcutaneous injection of 2 grams of sodium tartrate. They were allowed plenty of water and drank freely. On the day following the tartrate injection the animals were almost or completely anuric and were used for experimentation.

[8]Underhill, Wells and Goldschmidt: Journ. of Exper. Med., 1913, xviii, 322.

Experiment 27. Rabbit ♀, nephritic, weighing 3000 grams.

June 2—Placed in cage with plenty of water but without food.

June 4—Subcutaneous injection of 2 grams of *sodium tartrate.*

June 5—Weight, 2600 grams. Had drunk 140 cc. of water in the last twenty-four hours. Animal was anuric but had diarrhoea.

Injection of 130 cc. of Ringer's solution in 2 minutes 15 seconds.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	38	100
After injection		
Immediately	25	152
5 minutes	30	126
10 minutes	30	126
15 minutes	30	126
20 minutes	31	122
25 minutes	30	126
30 minutes	30	126
35 minutes	30	126
50 minutes	31	122
60 minutes	30	126

Injection II of 130 cc. of Ringer's solution in 3 minutes.

After injection		
Immediately	20	190
5 minutes	25	152

Rabbit died, with evident respiratory failure. Kidneys were pale, hypertrophied and watery; bladder was empty. Peritoneal cavity contained about 10 cc. fluid and there was a very little clear fluid in the pleural cavity. Some subcutaneous edema about abdominal region.

From the results of this experiment it is evident that tartrate nephritis has the same effect on the regulation of the blood volume shown in uranium and chromate nephritis. The severity of the nephritis is shown by the fact that even after injection of 260 cc. of Ringer's solution, no urine was secreted. Sixty per cent of the injected fluid left the circulation during the period of injection and 20 per cent during the next five minutes. However, 20 per cent of the solution introduced still remained in the blood stream, and no further removal of this excess of fluid took place in the following 50 minutes. Death, due to respiratory failure, resulted after a second injection.

The average figures obtained from all the experiments on normal and

nephritic rabbits are given in table 5 and these figures were used in plotting the curves shown in the accompanying diagram.

TABLE 5

Experiments on regulation of blood volume in rabbits

Average figures

	RELATIVE BLOOD VOLUMES AFTER							
	5 min.	10 min.	15 min.	20 min.	25 min.	30 min.	35 min.	40 min.
Normal	146	121	116	107	103	102	100	
Nephritic (uranium) Injection I	157	133	127	119	118	116	116	117
Nephritic (tartrate) Injection I	152	123	120	123	115	117	115	115
Nephritic (uranium) Injection II	187	149	129	129	127	127	125	
Nephritic (tartrate) Injection II	165	144	134	130	130	125	123	126

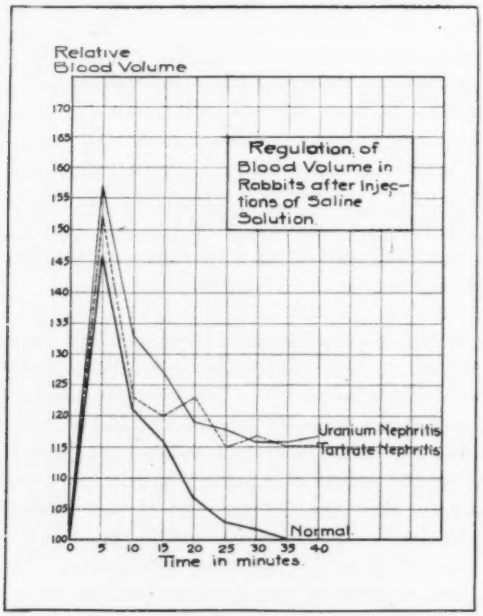

Fig. 3

Discussion of results. The experimental evidence given above, in conjunction with the work of Boycott ('13 and '14) and Chisholm ('14, a and b), shows clearly that fluid leaves the blood and passes into the tissues less readily in nephritic than in normal rabbits. It remains to be considered which of the factors concerned in the adjustment of the blood volume is so altered by the nephritis as to hinder the passage of fluid from the blood to the tissues. No change in the osmotic pressure of the blood would be caused by the injection of Ringer's solution and

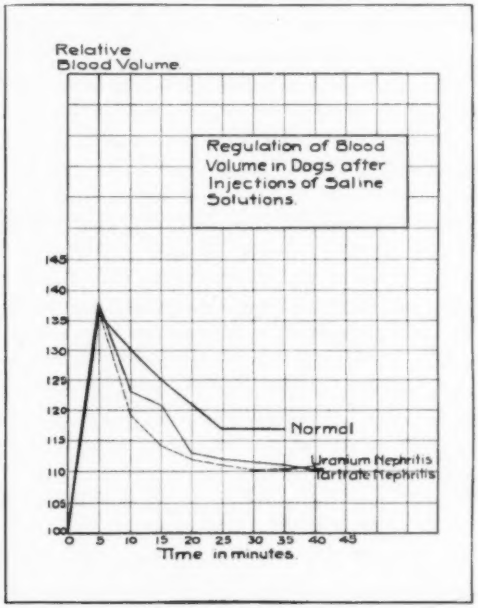

Fig. 4

the conditions of blood pressure would undoubtedly be equally affected in both the nephritic and normal animals. The experiments on rabbits with ligated kidneys and after food and water deprivation show that, with the experimental procedure employed in these investigations, the factors of kidney activity and condition of the tissues have little apparent effect on the regulation of the blood volume. The possibility of the retention of water by the nephritic animals causing a dilution and lowered osmotic pressure of the tissue fluids has not been excluded; but

this factor would seem to be more than balanced by the retention of solids in anuric conditions, especially as the nephritic animals frequently refused water. An alteration of the permeability of the blood vessel walls seems the only adequate explanation of the changed conditions prevailing in the nephritic animals. It is, therefore, concluded that the condition of nephritis effects an alteration of the permeability of the walls of the capillaries of the general circulation in such a way as to hinder the passage of fluid from the blood to the tissues.

To test the limits of tissue capacity. As was pointed out in the discussion of the experiments on rabbits with ligated kidneys, the injected saline solution does not leave the blood through the activity of the kidney nor is it to be found as transudates into the body cavities or in the alimentary canal. It must, therefore, be absorbed by the tissues. The following experiments were carried out with a view to ascertaining how much fluid a rabbit with ligated kidneys could dispose of and still keep the blood volume normal. It was also hoped to determine whether the tissues of nephritic rabbits showed a diminished capacity to hold water.

Experiment 70. Rabbit ♂, both kidneys ligated, weighing 2000 grams. Injection of 400 cc. of Ringer's solution in 9 minutes.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	61	100
After injection		
Immediately	37	165
5 minutes	51	120
20 minutes	58	105
30 minutes	61	100
40 minutes	61	100

Both kidneys found completely ligated; peritoneal cavity with 10 cc. clear fluid; pleural cavity with no fluid; walls of the alimentary tract highly edematous; no respiratory distress.

Experiment 67. Rabbit ♀, both kidneys ligated, weighing 2420 grams. Injection of 414 cc. of Ringer's solution in 7 minutes.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	58	100
After injection		
Immediately	24	242
10 minutes	32	181

Respiratory difficulty was so great that injection was discontinued before four times the blood volume (480 cc.) was injected. Dyspnoea continued unabated till death. On autopsy both kidneys were found completely ligated; peritoneal cavity with only 1 to 2 cc. of fluid; pleural cavity moist; lungs very congested but with no hemorrhages; viscera of the peritoneal cavity highly edematous.

Experiment 70 shows that a normal rabbit, with kidneys ligated, can dispose of a quantity of fluid equal to four times the calculated blood volume of the animal. A large amount of water is taken up by the walls of the intestines but the amount secreted into the intestinal lumen is small. There is no general edema, only a small quantity of fluid in the peritoneal cavity and none in the pleural cavity.

Experiment 67 shows that the limit of the capacity of the tissues is reached at approximately four times the blood volume of the animal, as in this experiment, this quantity, which was successfully disposed of in experiment 70, caused death when injected a little more rapidly. Injections of saline solution equal to two and three times the original blood volume (see table 6, experiments 66 and 68) were well borne, but death resulted when an injection equal to four times the blood volume was given to an animal, which had previously received a quantity of fluid equal to two times its blood volume. Death was preceded in each case by symptoms of great respiratory distress and the ejection of fluid from the nose. It is evident, from the figures in table 6, that, in order to have the animal dispose of the fluid, it is important that the rate of injection be so regulated that the liquid is not introduced much more rapidly than it can leave the circulation. Thus, in experiment 68, there was a greater initial dilution and more difficulty in disposing of the excess of fluid than in experiment 70, where the volume of the solution introduced was greater but where the rate of injection was slower.

Experiment 73. Rabbit ♀, nephritic, weighing 1680 grams.

December 10—Subcutaneous injection of 10 mg. of *uranium nitrate* per kilogram.

December 14—Urine for previous twenty-four hours, 35 cc. which gave a heavy precipitate on heating and faint reduction of Nylander's solution. Weight 1600 grams.

Injection of 320 cc. of Ringer's solution in 11 minutes.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	46	100
After injection		
Immediately	26	177
10 minutes	38	121
20 minutes	37	124
30 minutes	38	121
40 minutes	38	121
50 minutes	38	121
60 minutes	38	121

Kidneys were pale and edematous; bladder was empty; pleural cavity contained about 35 cc. of rapidly clotting, clear fluid, while pleural cavity contained about 3 cc. of clear fluid.

Experiment 74. Rabbit ♀, nephritic, weighing 1700 grams.

December 12—Subcutaneous injection of 10 mg. *uranium nitrate* per kilogram.

December 15—No urine voided in previous twenty-four hours.

December 16—Urine, 5 cc. containing sugar and protein. Animal used for experimentation.

Injection of 340 cc. of Ringer's solution in 12 minutes.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	48	100
After injection		
Immediately	24	200
10 minutes	34	141
20 minutes	35	137
30 minutes	35	137
40 minutes	35	137
50 minutes	35	137

Kidneys were very pale and inflamed, with much edema; bladder was empty; the pleural cavity contained 5 cc. and the peritoneal cavity 45 cc. of clear fluid which clotted rapidly; walls of the intestine edematous.

Experiment 73 furnishes evidence that the capacity of the tissues to hold water is no less in nephritic than in normal conditions. It was thought that the nephritic rabbits might not be able to dispose of large injections as well as of smaller ones, a greater excess of fluid remaining in the blood the larger the injection. In experiment 73, however, the relative blood volume at the end of the experiment is no higher than that usually seen after injections one-fourth as large. In experiment 74 the final relative blood volume is slightly higher but there is reason to conclude from the autopsy that the nephritis was of a more acute type than in experiment 73.

It has been shown that saline solutions equal to four times the blood volume can be completely removed from the circulation within thirty minutes in normal rabbits. In nephritic animals, under the same experimental procedure, the extent of fluid removal is equal to that observed when the amount of fluid introduced was equivalent to the blood volume. It is, therefore, concluded that the injection of saline solution employed in these experiments, that is, a volume equal to that of the blood, is sufficient to produce marked alterations in blood volume without introducing excessive quantities of fluid.

TABLE 6

Capacity of tissues in normal rabbits

	RELATIVE BLOOD VOLUME AFTER						
	Injection	10 min.	20 min.	30 min.	40 min.	50 min.	60 min.
Experiment 66. Two times the normal blood volume injected in 4 minutes	146	119	113	102	100	100	
Experiment 68. Three times the normal blood volume injected in 6 minutes	193	153	126	121	114	107	102
Experiment 70. Four times the normal blood volume injected in 9 minutes	165	120	105	100	100		
Experiment 67. Four times the normal blood volume injected in 7 minutes	242	181					

TABLE 7

Capacity of tissues in nephritic rabbits

	RELATIVE BLOOD VOLUME AFTER						
	Injection	10 min.	20 min.	30 min.	40 min.	50 min.	60 min.
Experiment 73. Four times the normal blood volume injected in 11 minutes	177	121	124	121	121	121	121
Experiment 74. Four times the normal blood volume injected in 12 minutes	200	141	137	137	137	137	

EXPERIMENTS WITH DOGS

In order to follow the lymph flow after intravenous injections of saline solutions, and to determine whether the above observations made on rabbits are applicable as well to dogs, the experiments made on normal and nephritic rabbits were repeated on dogs. Narcosis was produced by a subcutaneous injection of 10 mg. of morphine sulphate and 1 mg. of atropine sulphate per kilogram of body weight; and ether was given whenever necessary. A fistula of the thoracic duct having been made, the lymph was collected in five minute periods. Ringer's solution (25 cc. per kilogram) was injected, the remainder of the procedure being the same as in the experiments on rabbits.

Under normal conditions and after ligation of the kidneys. In experiment 18 the curve of the blood volume after saline injections is very different from that seen in normal rabbits. Instead of returning to normal within half an hour after the injection, the blood volume remains above normal throughout the experiment. A second injection produces a similar but more marked effect. Ligating the kidneys is without influence on the regulation of the blood volume.

The lymphagogic action of the saline injections is noticeable, the quantity of lymph being increased four fold or more. The amount of fluid which leaves the blood and passes into the thoracic lymphatic system is, however, small in proportion to the size of the injection. In experiment 18, after the injection of 500 cc. of Ringer's solution, the lymph collected amounted to only 123 cc. or about 25 per cent of the injected fluid. Most of the fluid had left the blood but was evidently absorbed and held back by the tissues.

Experiment 18. Dog ♂, normal, weighing 10.25 kilograms.

8.30 a.m.—Subcutaneous injection of 10 mg. morphine sulphate and 1 mg. atropine sulphate per kilogram.

9.30 a.m.—Anaesthetized with ether. Cannula in thoracic duct.

Injections of Ringer's solution into right femoral vein. Blood samples from left femoral artery.

REMARKS	LYMPH		BLOOD		RELATIVE BLOOD VOLUME
	Time	Interval flow	Time	Hemoglobin	
		cc.		*per cent*	
	10.41				
	10.46	1.5			
	10.51	1.65			
10.59–11.01, injection of 250 cc.	10.56	1.75	10.54	93	100
Ringer's solution	11.01	2.10	11.01	72	129
	11.03	5.4			
	11.07	9.9	11.06	77	121
Deep breathing and cries	11.12	10.6	11.11	80	116
	11.17	6.4	11.16	82	113
	11.22	5.6	11.21	84	111
	11.27	4.5	11.26	85	109
	11.32	4.0	11.31	84	111
	11.37	3.6	11.36	84	111
	11.42	3.9	11.41	84	111
	11.47	3.7			
	11.52	3.3	11.51	84	111
	11.57	2.8			
12.02–12.25, operation of ligating kidney at hilum	12.02	2.8	12.01	85	109
	12.25		12.30	86	108
12.30–12.32, injection of 250 cc.	12.30	1.3	12.32	68	137
Ringer's solution	12.35	2.1	12.37	75	124
	12.40	9.2	12.42	77	119
	12.45	6.7	12.47	79	117
	12.50	6.9	12.52	82	113
	12.55	5.6	12.57	80	116
	1.00	4.4	1.02	80	116
	1.05	4.3	1.07	82	113
	1.10	3.9	1 .12	83	112
Deep breathing and cries	1.15	3.7			
	1.20	3.4			
	1.25	2.8	1.22	87	107
	1.30	3.3			
	1.35	3.0	1.32	86	108

Experiment 26. Dog ♂, nephritic, weighing 12 kg.

June 2—Subcutaneous injection of 6.7 mg. *uranium nitrate* per kilo.

June 3—Urine 560 cc. Specific gravity 1.030. Reaction acid. Heavy flocculent precipitate on heating. Heavy sediment on standing. Very few casts, round cells very prominent, no blood cells. Benedict's solution strongly reduced.

Injection of 6.7 mg. *uranium nitrate* per kg.

June 4—Weight 10 kg. Drank freely of water. Urine 420 cc. Sediment on standing.

9 a.m.—Injection of 10 mg. morphine sulphate and 1 mg. atropine sulphate per kg.

10 a.m.—Anaesthetized with ether. Cannula inserted in thoracic duct.

REMARKS	LYMPH		BLOOD		RELATIVE
	Time	Interval flow	Time	Hemoglobin	Blood volume
		cc.		*per cent*	
	10.22				
	10.27	2.7	10.26	63	100
10.36–10.38 Injection I of 250	10.32	2.9			
cc. Ringer's solution	10.39		10.38	46	137
Lymph grows turbid	10.44	3.5	10.43	52	121
Lymph very turbid	10.49	3.7	10.48	55	114
	10.54	4.0	10.53	55	114
	10.59	4.8	10.58	56	112
Lymph watery and clear	11.04	5.0	11.03	57	111
	11.09	3.5	11.08	54	116
	11.14	2.9	11.13	55	114
11.23–11.24½ Injection II of 250	11.19	2.2	11.18	55	114
cc. Ringer's solution	11.25		11.25	42	150
Lymph clear	11.30	6.4	11.30	47	134
Lymph turbid	11.35	3.8	11.35	52	121
	11.40	4.0	11.40	54	116
	11.45	3.5	11.45	54	116
	11.50	3.6	11.50	54	116
	11.55	4.1	11.55	55	114
Lymph clear	12.00	3.1	12.00	55	114
	12.05	3.0	12.05	56	112
	12.10	4.3	12.10	56	112
	12.15	3.4	12.15	57	110
12.23–12.27 Injection III of 500	12.20	3.4	12.20	56	112
cc. Ringer's solution			12.27	40	157
	12.32	10.7	12.32	44	143
Lymph turbid	12.37	8.5	12.37	45	140
	12.42	6.8	12.42	46	137
	12.47	5.6	12.47	46	137
	12.52	5.6	12.52	48	133
	12.57	9.3	12.57	48	133
	1.02	4.2	1.02	49	128
	1.07	5.4	1.07	51	123
	1.13	4.6	1.13	52	121
	1.18	1.1			
	1.23	.6	1.21	50	126
No flow of lymph—due to wrong position of cannula—no clot.	1.28	1 drop	1.26	52	121

Kidneys were pale, hypertrophied and watery; bladder contained a little less than 50 cc. of urine; peritoneal cavity contained about 5 cc. of fluid.

In uranium nephritis and in tartrate nephritis. Uranium nephritis was induced by subcutaneous injections of 6.7 mg. of uranium nitrate per kilogram of body weight on two successive days and the dogs were used for experimental purposes on the third day.[9] Tartrate nephritis was produced in the same manner as in the rabbits.

Experiment 32. Dog ♀, nephritic, weighing 8.2 kg.

June 14—Placed in cage without food. Drank freely.

June 16—Subcutaneous injection of 8 g. *sodium tartrate.*

June 17—8.30 a.m. Subcutaneous injection of 10 mg. morphine sulphate and 1 mg. atropine sulphate per kilogram.

Anaesthetized with ether at 9.45 a.m. Cannula inserted in thoracic duct.

REMARKS	LYMPH		BLOOD		RELATIVE
	Time	Interval flow	Time	Hemoglobin	Blood volume
		cc.		*per cent*	
	10.57				
Lymph flow uniform	11.02	0.8	11.02	75	100
11.03–11.04 Injection I of 200	11.07	2.5	11.05	56	134
cc. Ringer's solution	11.12	7.1	11.10	63	121
	11.17	4.5	11.15	66	114
Lymph clears	11.22	4.2	11.20	68	110
	11.27	2.7	11.25	70	107
Animal cries	11.32	3.3	11.30	70	107
	11.37	2.5	11.35	69	108
11.43–11.44 Injection II of 200	11.42	2.2	11.40	70	107
cc. Ringer's solution	11.47	7.0	11.45	54	139
	11.52	14.0	11.50	60	125
	11.57	9.5	11.55	60	125
	12.02	7.0	12.00	62	121
	12.07	5.1	12.05	64	117
	12.12	5.2	12.10	66	114
	12.17	3.8	12.15	67	112
12.23–12.25 Injection III of 400	12.22	3.9	12.20	65	115
cc. Ringer's solution	12.27	12.0	12.25	43	174
	12.32	13.7	12.31	55	136
	12.37	8.1			
	12.42	8.4	12.40	63	121
	12.47	8.0	12.45	63	121
	12.52	8.4	12.50	63	121
Respiration increased	12.57	10.6	12.55	63	121
	1.02	8.5	1.00	65	115

Kidneys were pale but normal in size and without edema. Bladder contained 30 cc. of urine, which was very light in color and gave a strong protein test; there was no fluid in either the pleural or peritoneal cavities.

[9] MacNider: Journal of Med. Res., 1913, xxviii, 403.

These experiments display a condition similar to that in the normal dog and in the nephritic rabbit following saline injections. There is uniform inability to restore the blood volume to normal and it will be noted that the excess of fluid in the circulation is about the same in dogs after two injections of 25 cc. per kilogram as in nephritic rabbits after a single injection of 50 cc. per kilogram. The lymph flow varies widely in individual dogs but is not markedly different in nephritic and normal animals. These observations are shown in graphic form in the charts which follow.

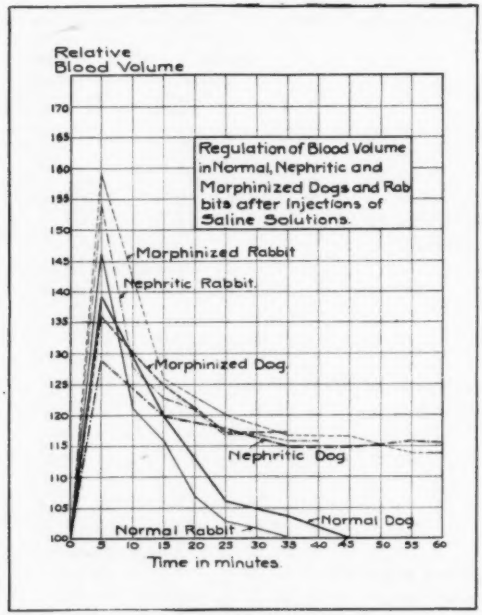

Fig. 5

THE INFLUENCE OF MORPHINE AND ATROPINE UPON BLOOD VOLUME REGULATION FOLLOWING INJECTIONS OF SALINE SOLUTIONS

In attempting to explain these conflicting data, it seemed hardly probable that difference in species would account for this wide divergence in physiological behavior. The most important distinction in experimental procedure appeared to be that the dogs were given a subcuta-

neous injection of 10 mgm. of morphine sulphate and one of atropine sulphate per kilogram one hour previous to anaesthetization, while the rabbits were not. Investigations were, therefore, made to determine whether this factor was responsible for the different results obtained in the experiments on dogs.

In normal dogs. The results of such experiments are seen in the following illustrative protocol.

Experiment 71. Dog ♂, normal, weighing 8 kg. No morphine and atropine injection given. Animal anaesthetized with ether. Injection of 200 cc. of Ringer's solution in 1 minute 30 seconds.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	80	100
After injection		
Immediately	61	131
10 minutes	70	114
20 minutes	75	107
30 minutes	80	100

Experiment 71 demonstrates that normal dogs which have not received morphine and atropine are able to completely restore the blood volume to normal after saline injections, behaving in the same manner as rabbits under similar circumstances. This is confirmed by the results of four other similar experiments. On the other hand, Experiments 43 and 65 (see table 2) argue against this conclusion, since, in these experiments, the blood volume had not returned to normal after periods of forty minutes and one hour respectively, although the dogs had received no morphine-atropine injection and were supposedly normal. As no proof was obtained that these animals were actually normal and as one of them (experiment 43) was obviously in very poor condition, a fact which was further evidenced by the abnormally low content of hemoglobin and total solids in the blood, it is possible that they were either spontaneously nephritic or were suffering from some other condition which produces a similar effect on the regulation of the blood volume. The fact that none of the animals, which received morphine and atropine were able to completely restore their normal blood volume after saline injections, while this was accomplished by five dogs, which had not received morphine-atropine injections, clearly

indicates that this factor is responsible for the inability to restore normal blood volume.

In normal rabbits. In order to complete the evidence on this point, three rabbits were given injections of morphine and atropine sulphates, the same amounts per kilogram of body weight being subcutaneously administered and the same length of time being allowed to intervene before the injection of the saline solution as in the experiments on dogs. The rabbits showed themselves less sensitive to morphine than the dogs, for the narcotic effect of that drug was not noticeable. Nevertheless, the effect on blood volume after injections of saline solution was the same as that observed in dogs. This is readily seen in the protocol which follows.

Experiment 63. Rabbit ♂, with morphine and atropine injection. Weight, 1800 grams.

9 a.m.—Subcutaneous injection of 10 mg. morphine sulphate and 1 mg. atropine sulphate per kilogram. 10 a.m.—Bladder emptied and animal etherized. Injection of 90 cc. of Ringer's solution in 1 minute 30 seconds.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	49	100
After injection		
Immediately	29	169
10 minutes	40	122
20 minutes	43	114
30 minutes	42	114
40 minutes	42	114
50 minutes	43	114
60 minutes	42	114

Bladder contained 5 cc. of urine; no fluid in the pleural cavity; 2 to 3 cc. fluid in the peritoneal cavity.

The subcutaneous injection of morphine and atropine sulphates results, therefore, in an ineffective regulation of the blood volume similar to that produced by nephritis. It seems probable that the morphine-atropine injection decreases the permeability of the capillary walls either directly by acting on the blood vessels themselves or indirectly by producing some injury to the kidney which results in this decreased capillary permeability. Whether this effect is due to the morphine or to the atropine or to their combined action, remained to be determined and the results of this investigation are seen in the following experiments.

Experiment 79. Rabbit ♂, with atropine injection, weighing 2220 grams.
1.15 p.m.—Subcutaneous injection of 1 mg. atropine sulphate per kilogram.
2.20 p.m.—Animal anaesthetized with ether.
Injection of 110 cc. of Ringer's solution in 2 minutes 10 seconds.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	66	100
After injection		
Immediately	48	137
10 minutes	58	114
20 minutes	66	100
30 minutes	66	100

Experiment 80. Rabbit ♂, with morphine injection, weighing 2420 grams.
2.20 p.m.—Subcutaneous injection of 10 mg. morphine sulphate per kilogram.
3.20 p.m.—Anaesthetized with ether. The most marked narcotic effect of morphine noted in rabbits was seen in this experiment. The animal was rendered stupid before ether was given and after anaesthetization no further ether was required in the course of an hour.
Injection of 120 cc. of Ringer's solution in 2 minutes 20 seconds.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	69	100
After injection		
Immediately	48	144
10 minutes	58	119
20 minutes	61	113
30 minutes	61	113
40 minutes	62	111
50 minutes	62	111
60 minutes	62	111

Experiments 79 and 80 prove that the failure of the blood volume to return to normal, after injections of saline solutions, produced in both dogs and rabbits by the injection of mophine and atropine sulphates, is the result of the action of the morphine sulphate.[10] Experiment 80

[10] Since the above statement was written, Kleiner and Meltzer (Proc. Soc. Exp. Biol. and Med., 1916, xiii, 142) have indicated that, after intravenous injections of dextrose, morphine retards the disappearance of the excess of sugar from the circulation, as shown by a persistent hyperglycemia.

gives evidence that this effect of the morphine is not due to the decreased blood pressure, which it causes, since, in that event, the effect should be more pronounced when morphine is given alone than when it is given with atropine, which tends to nullify the action of the morphine on blood pressure.

In nephritic dogs. Experiments have been cited to show that the regulation of the blood volume after injections of saline solution is the same in normal dogs and rabbits without morphine administration. Injections of morphine have been shown to cause inability to restore the original blood volume after injections of saline solution in both dogs and rabbits. The same effect has been produced in rabbits by inducing a nephritic condition. If this partial recovery of original blood volume after injection were found to be the result of a nephritic condition in dogs, the analogy would be complete and the deductions made from experiments on one species might be regarded as applicable to the other. As, unfortunately, all the earlier data on nephritic dogs were taken from animals which had received morphine, no conclusions could be drawn from these experiments. A number of dogs were, therefore, rendered nephritic with uranium nitrate and injected with Ringer's solution as in the earlier procedure except that no morphine-atropine injection was given. When these animals were used for experimentation on the second day of the nephritis, little or no defect in the regulation of the blood volume was found. In all later experiments, therefore, the dogs were used on the fourth day of the nephritis, as was the case with the rabbits with uranium nephritis, and results entirely comparable with those noted in nephritic rabbits were obtained. A specimen protocol follows.

Experiment 77. Dog, nephritic. Weight 10.5 kg.—male.
January 18—Subcutaneous injection of 6.7 mg. *uranium nitrate* per kilogram.
January 19—Subcutaneous injection of 6.7 mg. *uranium nitrate* per kilogram.
320 cc. urine, containing sugar but no protein.
January 20—560 cc. urine, containing sugar and protein.
January 21—220 cc. urine, containing sugar and protein.
January 22—140 cc. urine, containing neither sugar nor protein.
Injection of morphine and atropine omitted. Ether anaesthesia only.
Injection of 250 cc. of Ringer's solution in 1 minute 50 seconds.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	72	100
After injection		
Immediately	56	128
10 minutes	59	122
20 minutes	62	116
30 minutes	62	116
40 minutes	64	113
50 minutes	62	116
60 minutes	62	116

Kidneys were congested, with great edema; bladder was empty; there was no fluid in the body cavities.

It thus appears that the existence of a condition of acute nephritis results in an inability of the animal to completely restore its blood volume to normal after injections of saline solution, in dogs as well as in rabbits.

In one experiment, although the characteristic effect of the nephritis on the regulation of the blood volume was present, it was much less marked than usual. This is probably accounted for by the fact that the dog used was young.[11] It seems somewhat more difficult to produce an acute nephritis in dogs than in rabbits. The diuretic effect of the uranium is more marked and anuria does not set in till later. While

TABLE 8

Regulation of blood volume in normal, nephritis and morphinized dogs and rabbits after injections of saline solution

Average figures

	RELATIVE BLOOD VOLUMES AFTER											
	Injection	5 min.	10 min.	15 min.	20 min.	25 min.	30 min.	35 min.	40 min.	45 min.	50 min.	60 min.
Normal rabbits	146	121	116	107	103	102	100					
Normal dogs	139		120		106		104		100		100	100
Nephritic rabbits	154	128	123	121	117	117	116	116				
Nephritic dogs	129		120		118		115		115		116	115
Morphinized rabbits	159		126		120		117		117		114	114
Morphinized dogs	136	130	125	121	117	117						

[11] MacNider, Journal of Pharmacology and Experimental Therapeutics, iv, 491, 1913.

it is not necessary that anuria should set in before the deficient regulation of the blood volume is noted, it is essential that the nephritis should have progressed to a certain degree of acuteness.

It seems probable, therefore, that, since the regulation of the blood volume has been shown by experimentation to be the same in both dogs and rabbits in all cases where similar conditions prevailed, conclusions drawn from experiments on either species may be considered as applicable to the other species.

SUMMARY OF THE MORE IMPORTANT CONCLUSIONS

The regulation of the blood volume in normal animals is both rapid and efficient; for complete restoration of the original blood volume takes place within thirty minutes after the intravenous injection of a quantity of saline solution equal to the calculated blood volume of the animal.

The activity of the kidneys is not essential to this regulation of the blood volume, as it occurs even after ligation of these organs.

In conditions of nephritis, inability to restore the blood volume to normal after injections of saline solution usually manifests itself. Experiments on animals with *uranium nephritis* confirm the results of Boycott in this respect. New experiments on animals with *nephritis induced by sodium tartrate* reveal a similar incomplete regulation of the blood volume under these circumstances.

Morphine sulphate, administered subcutaneously in large doses, *produces an effect on the regulation of the blood volume* comparable with that resulting from a condition of nephritis.

The factor of species is probably of little importance in studies on this regulation of the blood volume, since dogs and rabbits react alike when the conditions are identical.

Restoration of the blood volume to normal after intravenous injections of saline solutions is not primarily effected by the transfer of the excess of fluid from the blood vessels to the thoracic lymphatic system. The tissues act as a reservoir for this fluid.

The capacity of the tissues of rabbits to absorb fluid is approximately four times the normal blood volume of the animal.

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CHISHOLM: Ibid , 1914(b), xix, 265.

THE ACTION OF SALINE-COLLOIDAL SOLUTIONS UPON THE REGULATION OF BLOOD VOLUME[1]

Studies of the Permeability of Cellular Membranes. II

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Received for publication, June 14, 1916

There are few references in the literature relative to intravenous injections of colloidal solutions. In explanation of the small changes in arterial pressure caused by the injection of large quantities of blood, von Regéczy ('85) offered the suggestion that the injected fluid leaves the circulation. As proof of this, he cited an increased lymph flow following such injections and the higher specific gravity of the blood after blood of a lower specific gravity had been injected. Magnus ('01) also made large injections of blood and reported that 30 to 56 per cent of the injected fluid left the circulation within an hour although no diuresis resulted. Roth ('99) found that, when a protein solution (dialyzed horse serum) was placed in the peritoneal cavity, water was first drawn into the blood on account of the low osmotic pressure of the protein solution, but that later, when the osmotic pressures were equalized, protein began to diffuse into the blood and salts to diffuse out. He concluded that water in the form of a colloidal solution can pass through the walls of the blood vessels and that these walls are semipermeable for colloids as well as crystalloids.

Fischer and Hogan ('12) made intravenous injections of gelatin solutions and reported that these were not diuretic and that, therefore, high arterial pressure was maintained for a longer time after such injections than after injections of saline solutions. It was suggested that gelatin solutions might advantageously replace ordinary saline solutions for injection in cases of shock where it is desirable to keep the blood pressure high for a considerable period of time (Hogan '15). In the ex-

[1] The data in this paper are taken from the dissertation presented by L. Jean Bogert for the degree of Doctor of Philosophy, Yale University, 1916.

periments of Roger and Garnier ('13) Locke's solution, to which gum or gelatin had been added, was found to leave the blood vessels less readily than plain Locke's solution. That gelatin solutions disappear from the blood stream less rapidly than saline solutions and less rapidly in nephritic than in normal rabbits were the conclusions reached by Boycott ('14) as a result of his experiments on the injection of colloidal solutions in rabbits.

EXPERIMENTAL

The experiments to be detailed were carried out in the same manner as those in the preceding paper except that the Ringer's solution injected contained approximately 2 per cent of gelatin. In making this solution care was taken to follow the directions given by Hogan ('15) for making gelatin solutions for injection in man and evidently the colloidal nature of the gelatin was not injured.

EXPERIMENTS WITH RABBITS

Under normal conditions. Of the six normal rabbits, which received injections of gelatin solution, three were able to restore their blood vol-

Experiment 50. Rabbit ♀, normal, weighing 1700 grams. Injection of 85 cc. of gelatin solution in 2 min. 5 sec.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	43	100
After injection		
Immediately	30	143
5 minutes	34	126
10 minutes	38	113
15 minutes	39	110
20 minutes	39	110
25 minutes	39	110
35 minutes	41	105
40 minutes	43	100
45 minutes	43	100
50 minutes	43	100
60 minutes	43	100
70 minutes	43	100

In the bladder were 50 cc. of urine, secreted during the experiment, which contained no coagulable protein but gave a strong biuret test. There were about 4 cc. of fluid in the peritoneal cavity and a small amount in the pleural cavity.

ume to normal in the same length of time as after a saline injection, or with only a slight delay, while three rabbits showed some difficulty in disposing of the injected fluid, from 11 to 27 per cent of it being still present in the circulation after an hour. Experiment 50 is an example of the first group and experiment 46 represents the second group.

Experiment 46. Rabbit ♀, normal, weighing 3100 grams. Injection of 150 cc. of gelatin solution in 4 min. 20 sec.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	63	100
After injection		
Immediately	38	166
5 minutes	39	161
10 minutes	42	150
15 minutes	41	154
20 minutes	48	131
25 minutes	48	131
30 minutes	50	126
35 minutes	50	126
40 minutes	52	121
45 minutes	54	117
50 minutes	54	117
60 minutes	55	115
70 minutes	54	117

In the bladder were 30 cc. of urine, containing no coagulable protein or reducing substances but giving a strong biuret test. There was no fluid in the pleural cavity and only 3 to 4 cc. in the peritoneal cavity.

The bladder was emptied previous to the injection, in these experiments, and the urine secreted during the period of observation was collected. The amount secreted in one hour after the injection varied from 6 to 59 per cent of the injected fluid, with an average figure of 27 per cent. The data collected on urine excretion in normal rabbits after saline injections show that 9 to 53 per cent of the injected fluid, or an average of 28 per cent was excreted by the kidneys in the hour following the injection. From these experiments, therefore, it appears that after introduction of colloidal solutions fluid is excreted to the same extent as with saline solutions.

Boycott ('14) found that gelatin leaves the circulation and that the quantity lost varies, in a general way, with the quantity of liquid expelled. In the above experiments, the samples of urine taken an hour

after the injection were all found to show no coagulable protein but to give a strong biuret test. This indicates that not only can the gelatin leave the blood stream but it can be excreted by the kidneys as well.

In nephritis. There is also considerable difference in the relative ease with which the nephritic rabbits disposed of the colloidal injections. Experiment 52 shows a case where the colloidal solution left the circulation with no greater difficulty than a saline solution. In experiment 56, however, a large proportion of the solution was unable to leave the blood vessels during the experiment. The amount of the injected fluid still in the circulation after one hour varied from 11 to 57 per cent.

Experiment 52. Rabbit ♀, nephritic weighing 2600 grams.

October 15—Subcutaneous injection of 10 mg. *uranium nitrate* per kilogram.

October 19—Urine for previous twenty-four hours, 6 cc., which contains sugar and protein. Animal in good condition.

Injection of 125 cc. of gelatin solution in 3 minutes.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	43	100
After injection		
Immediately	34	126
5 minutes	35	122
10 minutes	36	119
15 minutes	38	113
20 minutes	38	113
25 minutes	36	119
30 minutes	38	113
35 minutes	38	113
40 minutes	38	113
45 minutes	39	110
50 minutes	38	113
60 minutes	38	113

Kidneys were very pale, with marked edema; bladder was empty; pleural and peritoneal cavities each contained 2 to 3 cc. of clear fluid.

Experiment 56. Rabbit ♂, nephritic, weighing 1980 grams.
October 19—Subcutaneous injection of 10 mg. *uranium nitrate* per kilogram.
October 23—Animal used for injection of gelatin solution.
Injection of 98 cc. of gelatin solution in 2 minutes 25 seconds.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	51	100
After injection		
Immediately	30	170
5 minutes	34	150
10 minutes	35	146
15 minutes	35	146
20 minutes	34	150
25 minutes	35	146
30 minutes	35	146
35 minutes	35	146
40 minutes	35	146
45 minutes	35	146
50 minutes	35	146
60 minutes	35	146
70 minutes	35	146

Kidneys were pale and enlarged, with marked edema; in the bladder were 43 cc. of light, turbid urine, containing a small amount of sugar and coagulable protein and giving a strong biuret test; there were 4 cc. of clear fluid in the peritoneal cavity while the pleural cavity contained no fluid.

The average figures obtained from the experiments with injections of colloidal solution in normal and nephritic rabbits are given in table 9 and these data were used in plotting the curves in the accompanying chart.

TABLE 9

Regulation of the blood volume after injections of saline-colloidal solution

Average figures

	RELATIVE BLOOD VOLUMES AFTER												
	Injection	5 min.	10 min.	15 min.	20 min.	25 min.	30 min.	35 min.	40 min.	45 min.	50 min	60 min.	70 min.
Normal rabbits—Group I	173	134	123	117	117	117	111	107	103	103	102	100	100
Normal rabbits—Group II	170	157	146	142	130	127	125	124	122	120	121	117	118
Nephritic rabbits	152	143	138	136	136	136	135	135	134	133	134	135	

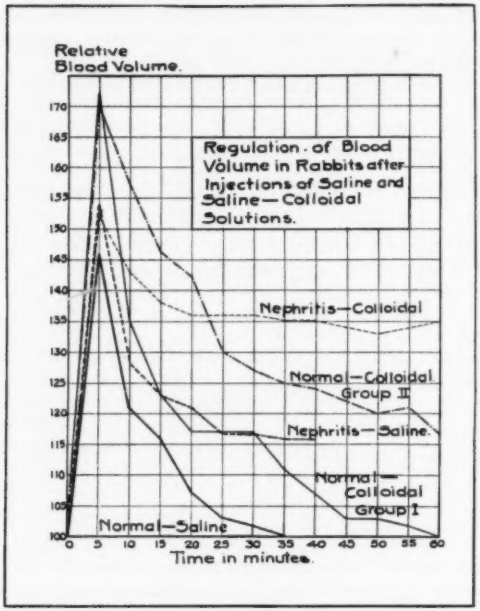

Fig. 1

EXPERIMENTS WITH DOGS

Under normal conditions and in nephritis. Both the normal and nephritic dogs disposed of the gelatin injections with ease. As morphine-atropine injections were given in each experiment, the animals were never able to completely restore their blood volume to normal but they did so to as great or greater extent than after saline injections. The colloidal injections showed a marked lymphagogic action. The average amount of the injected fluid collected as lymph is 25 per cent in the normal dogs and 34 per cent in the nephritic dogs. These figures are slightly larger than corresponding figures after saline injections. These animals, therefore, had no difficulty in expelling from the circulation excess of fluid held in colloidal solution. The protocols which follow show the course of two such experiments and the general results are shown in the accompanying charts.

Experiment 34. Dog ♂, normal, weighing 13 kg.

8.30 a.m.—Subcutaneous injection of 10 mg. morphine sulphate and 1 mg. atropine sulphate per kilogram.

9.30 a.m.—Anaesthetized with ether. Cannula in thoracic duct.

REMARKS	LYMPH		BLOOD		RELATIVE BLOOD VOLUME
	Time	Interval flow	Time	Hemoglobin	
		cc.		*per cent*	
	10.03		10.03	80	100
10.10–10.12 Injection I of 325	10.08	1.7			
cc. gelatin solution	10.13	1.2	10.12	60	133
	10.18	7.9	10.17	65	123
	10.23	10.1	10.22	67	118
	10.28	8.5	10.27	69	116
	10.33	6.5	10.32	72	111
	10.38	5.5	10.37	72	111
	10.43	5.0	10.42	71	113
10.50–10.52 Injection II of 325	10.48	4.1	10.47	71	113
cc. gelatin solution	10.53	3.6	10.52	57	140
	10.58	17.7	10.57	60	133
	11.03	14.5	11.02	60	133
	11.08	11.5	11.07	62	129
	11.13	10.8	11.12	65	123
	11.18	10.5	11.17	68	118
	11.23	10.2	11.22	68	118
	11.28	10.8	11.27	68	118
	11.33	9.5			
11.39–11.43 Injection III of 650	11.38	10.4			
cc. gelatin solution	11.43	10.0	11.43	50	160
	11.48	38.5	11.48	52	154
	11.53	36.6	11.53	53	151
	11.58	26.0	11.58	56	143
	12.03	23.1	12.03	57	140
	12.08	21.0	12.08	59	135
	12.13	22.6	12.13	58	138
	12.18	18.5	12.18	62	129

There was no fluid in either of the body cavities; in the bladder were 100 cc. of medium colored urine which contained no coagulable protein but gave the biuret test.

Experiment 59. Dog ♂, nephritic, weighing 10 kg.

October 25—Subcutaneous injection of 6.7 mg. *uranium nitrate* per kg.

October 26—Subcutaneous injection of 6.7 mg. *uranium nitrate* per kg. Urine 200 cc. Drank freely.

October 27—Anuric. Drank freely. Weight 10 kg.

8.30 a.m.—Injection of 10 mg. morphine sulphate and 1 mg. atropine sulphate per kilogram.

9.30 a.m.—Anaesthetized with ether. Cannula in thoracic duct.

REMARKS	LYMPH		BLOOD		RELATIVE BLOOD VOLUME
	Time	Interval flow	Time	Hemoglobin	
		cc.		*per cent*	
10.35–10.37 Injection I of 250 cc. gelatin solution	10.30		10.25	82	100
	10.35	2.5	10.37	66	124
	10.40	4.6	10.42	69	119
	10.45	8.7	10.47	71	115
	10.50	7.9	10.52	74	111
	10.55	7.9	10.57	75	109
	11.00	7.8	11.02	75	109
	11.05	7.5	11.07	75	109
	11.10	7.7	11.12	75	109
11.16–11.18 Injection II of 250 cc. gelatin solution	11.15	7.6	11.18	58	141
	11.20	10.2	11.23	63	130
	11.25	20.7	11.28	67	122
	11.30	15.2	11.33	70	117
	11.35	13.4	11.38	70	117
	11.40	12.2	11.43	70	117
	11.45	12.3	11.48	70	117
	11.50	12.8	11.53	70	117
11.57–12.00 Injection III of 500 cc. gelatin solution	11.55	11.1			
	12.00	14.6	12.00	51	161
	12.05	46.8	12.05	57	144
	12.10	35.2	12.10	59	139
	12.15	24.0	12.15	62	132
	12.20	20.8	12.20	65	126
	12.25	16.5	12.25	65	126
	12.30	16.1	12.30	65	126
	12.35	15.3	12.35	65	126

Kidneys were dark and congested, with no edema; in the bladder were 4 cc. of urine which contained coagulable protein; there were 10 cc. of fluid in the peritoneal cavity and none in the pleural cavity.

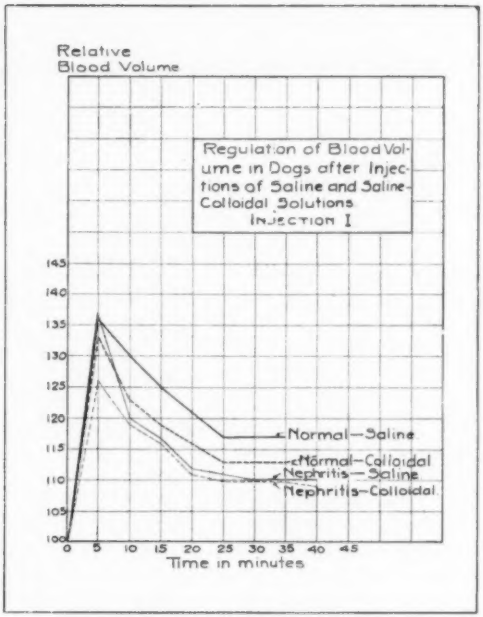
Relative
Blood Volume
Regulation of Blood Volume in Dogs after Injections of Saline and Saline-Colloidal Solutions
INJECTION I
145
140
135
130
125
120
115
110
105
100
0 5 10 15 20 25 30 35 40 45
Time in minutes
Normal—Saline
Normal—Colloidal
Nephritis—Saline
Nephritis—Colloidal

Fig. 2

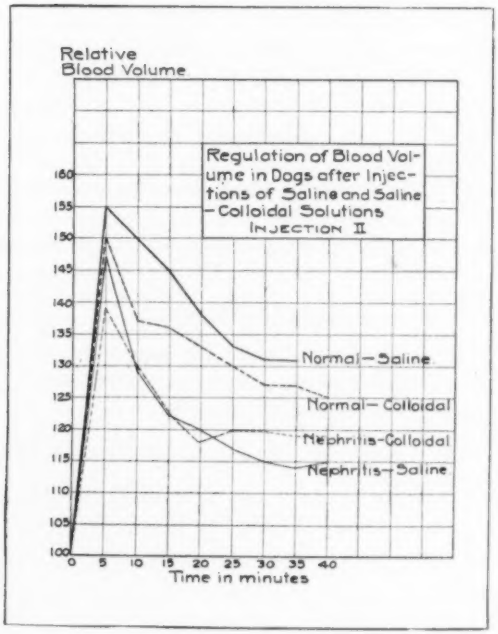
Relative
Blood Volume
Regulation of Blood Volume in Dogs after Injections of Saline and Saline-Colloidal Solutions
INJECTION II
160
155
150
145
140
135
130
125
120
115
110
105
100
0 5 10 15 20 25 30 35 40
Time in minutes
Normal—Saline
Normal—Colloidal
Nephritis-Colloidal
Nephritis—Saline

Fig. 3

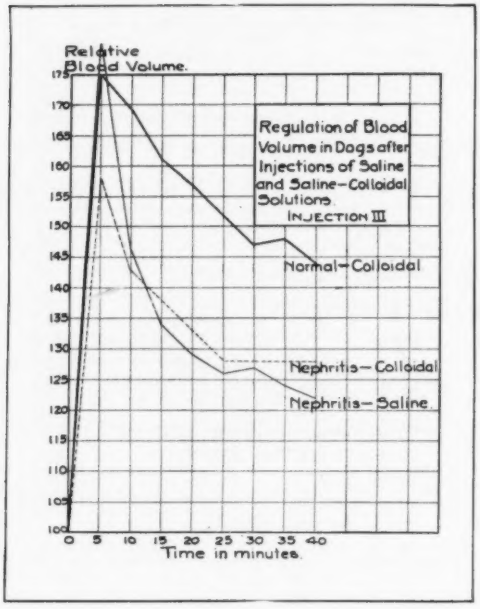

Fig. 4

CONCLUSION

Fluid injected in the form of colloidal solution *may* leave the circulation as readily as that in non-colloidal solution; but *usually the regulation of the blood volume is less efficient after the injection of colloidal solutions.*

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THE INFLUENCE OF ALKALINE-SALINE SOLUTIONS UPON REGULATION OF BLOOD VOLUME[1]

Studies of the Permeability of Cellular Membranes. III.

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Received for publication, June 14, 1916

The injection of alkaline solutions having been proposed by Fischer ('15)[2] as a means of dehydrating the colloids of the body, it was thought of interest to determine whether the regulation of the blood volume after injections is any different, especially in nephritic animals, when alkali is added to the injection fluid.

Sodium carbonate was dissolved in 0.9 per cent sodium chloride solution, as Ringer's solution could not be used on account of the precipitation of the calcium as calcium carbonate. Solutions equivalent to 0.2 per cent and 0.4 per cent sodium carbonate were injected,[3] the size of the injection being in each case equal to the calculated blood volume of the animal. The effect of such injections is seen in the following specimen protocols.

[1] The data in this paper are taken from the dissertation presented by L. Jean Bogert for the degree of Doctor of Philosophy, Yale University, 1916.

[2] Fischer, Oedema and Nephritis, Edition 2, John Wiley and Sons, 1915.

[3] For description of the technique of these experiments see this Journal, 1916, xli, 189.

Experiment 41. Rabbit ♀, normal, weighing 3360 grams. Injection, in 3 minutes 25 seconds, of 165 cc. of *0.2 per cent sodium carbonate* in 0.9 per cent sodium chloride solution.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	51	100
After injection		
Immediately	36	141
5 minutes	43	119
10 minutes	44	115
15 minutes	47	108
20 minutes	49	104
25 minutes	51	100
30 minutes	51	100

Bladder contained 38 cc. of urine, secreted during the experiment, which gave a very faint test for protein; animal was pregnant; there were 6 cc. of fluid in the peritoneal cavity and none in the pleural cavity.

Experiment 42. Rabbit ♀, normal, weighing 1680 grams. Injection, in 3 minutes 15 seconds, of 85 cc. of *0.4 per cent sodium carbonate* in 0.9 per cent sodium chloride solution.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	57	100
After injection		
Immediately	35	163
5 minutes	44	129
10 minutes	49	116
15 minutes	51	112
20 minutes	54	105
25 minutes	57	100
30 minutes	57	100

Bladder contained 17 cc. of urine, secreted during the experiment, which gave tests for protein; there were 2 cc. of fluid in the peritoneal cavity and none in the pleural cavity.

Experiment 45. Rabbit ♂, nephritic, weighing 1800 grams.

August 18—Subcutaneous injection of 10 mg. *uranium nitrate* per kilogram.

August 19—Urine, 145 cc.

August 20—Urine, 50 cc., thick and turbid.

August 21—Urine, 27 cc., strong tests for both protein and sugar.

Injection, in 4 minutes, of 90 cc. of *0.4 per cent sodium carbonate* in 0.9 per cent sodium chloride solution.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	41	100
After injection		
Immediately	24	171
5 minutes	31	132
10 minutes	31	132
20 minutes	31	132
25 minutes	32	128
30 minutes	32	128

Bladder was empty; kidneys were very pale and inflamed but with no edema; the pleural and peritoneal cavities each contained 1 to 3 cc. of fluid.

Experiment 49. Rabbit ♀, nephritic, weighing 2600 grams.

October 11—Subcutaneous injection of 10 mg. *uranium nitrate* per kilogram.

October 15—Urine, 10 cc. Sugar and protein both present. Animal lively and in excellent condition. Weight, 2540 grams.

Injection, in 2 minutes 5 seconds, of 125 cc. of *0.4 per cent sodium carbonate* in 0.9 per cent sodium chloride solution.

TIME	HEMOGLOBIN	RELATIVE BLOOD VOLUME
	per cent	
Before injection	53	100
After injection		
Immediately	34	156
5 minutes	41	129
10 minutes	42	126
15 minutes	45	117
20 minutes	45	117
25 minutes	45	117
30 minutes	45	117

Bladder was empty; kidneys were pale and inflamed; there were 7 cc. of fluid in the peritoneal cavity and none in the pleural cavity.

TABLE 10

Regulation of blood volume after injections of saline and alkaline-saline solutions

Average figures

	RELATIVE BLOOD VOLUMES AFTER						
	Injection	5 min.	10 min.	15 min.	20 min.	25 min.	30 min.
Normal rabbits							
Saline injection	146	121	116	107	103	102	100
Alkaline-saline injection	152	119	111	107	104	101	100
Nephritic rabbits							
Saline injection	154	128	123	121	117	117	116
Alkaline-saline injection	171	133	132	123	123	122	122

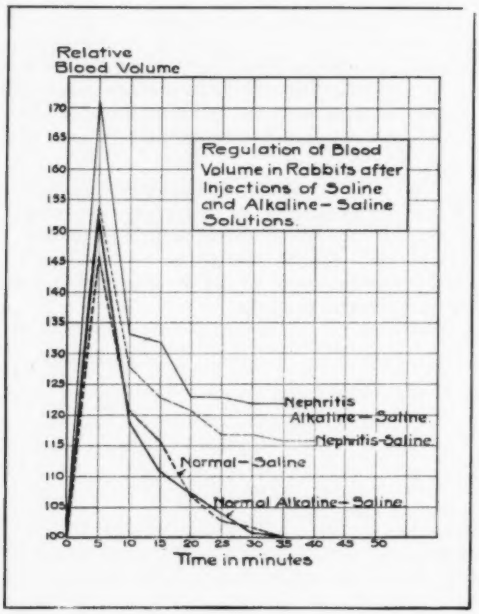

Fig. 1

CONCLUSION

It will be seen from these experiments and the accompanying chart, that *the addition of alkali*, up to 0.4 per cent sodium carbonate, to the fluid injected, *has no apparent effect on the regulation of the blood volume* either in normal or in nephritic animals. The rate at which, and the extent to which, injected fluid leaves the blood vessels is unaffected by the addition of sodium carbonate.

THE INFLUENCE OF IODIN AND SODIUM IODID ON THE CIRCULATION

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Received for publication, June 15, 1916

Iodin and its compounds have long been recognized among the best remedies in the possession of the physician. Their pharmacological action, however, is still imperfectly understood. This seems to be particularly true of their effect on the circulation, as shown by Capps (1), in a review of the literature on the subject. Some observers seem to believe that they are circulatory depressants while others brought forward evidence indicating that iodin and sodium iodid were, on the contrary, circulatory stimulants. According to Prevost and Binet (2) a fall of blood pressure is caused after intravenous injections into animals, but their effect is different when given by mouth. They maintained that iodin caused a rise of blood pressure, while the iodid failed to produce any effect on the circulation when they were administered by mouth.

Lehndorff (3) stated that sodium iodid injected intravenously into cats was followed by a rise of blood pressure amounting to 50 per cent, which he claimed was due to the increased cardiac output he observed as moderate dilatation of the peripheral arteries occurred at the same time. Macht (4) studied the action of iodin and iodid on the isolated heart of frogs and mammals as well as on intact animals and concluded that both of these substances are cardiac stimulants. His tracings show only a moderate increase in amplitude, but there was pronounced augmentation in the frequency of heart action. He also reported that sodium iodid caused moderate stimulation of the circulation in intact animals, but he failed to obtain any action with iodin.

That the behavior of sodium iodid varies with the condition of the circulation was maintained in a recent communication by Retzlauf (5). He injected intravenously a hypertonic solution of sodium iodid into rabbits whose blood pressure had been lowered by hemorrhage. The

first dose showed a marked but transient rise of blood pressure. The effect of successive doses given later diminished with the progressive rise of blood pressure.

In the course of studies on the action of various organic derivatives of iodin, we noticed that they often caused a marked fall of blood pressure. This suggested to us the advisability of testing the effect of molecular iodin and of sodium iodid on the circulation. The results form the subject of the present communication.

Iodin was dissolved in alcohol and was added to Ringer's solution, the alcohol being employed in order to facilitate the solution of iodin. The concentration of alcohol in the perfusion fluid varied between 0.03 and 0.16 per cent. It might be stated in advance, that this had no effect on cardiac action. Iodin was used in the proportions of 1 : 30,000; 1 :15,000; 1 :10,000; and 1 :5,000, these solutions being perfused for variable periods of time. In some experiments small quantities of olive oil were added to the Ringer-iodin solution. Studies were also made with different concentrations of sodium iodid.

The experiments were conducted on the isolated frog heart suspended in a moist chamber. The perfusing fluids were supplied by reservoirs in which a constant pressure was maintained. By means of rubber tubes these were connected with a four-way stopcock communicating with a cannula in the venous sinus. The heart could then be perfused with any of the fluids contained in the reservoirs. For a detailed description of the apparatus, the reader is referred to a recent publication from this laboratory (6). Experiments with iodin and sodium iodid were also carried out on intact animals, alcohol—chloretone or ether anesthesia being used.

The results of our observations with various concentrations of iodin indicate that iodin may produce disturbance of cardiac activity. Heart action became irregular, relaxation was incomplete, rate and amplitude varied. Periods of normal action alternating with these changes were sometimes also observed. With stronger solutions, complete suspension of activity occurred and lasted one-half to one minute. In nearly every case, depressing after effects which occasionally became very pronounced, were also observed. Some of these changes could be induced with dilute concentrations of iodin even in the fresh heart, which was found to be more resistant to poisons than at later stages of the experiment.

EXPERIMENTS WITH 1:30,000 IODIN

The results obtained indicated that a concentration of 1:30,000 iodin may cause disturbance of heart action, but the effect was never very pronounced and varied considerably. Thus, in one experiment in which perfusion with iodin lasted two minutes, a decrease of amplitude with a corresponding increase in rate were observed within one-half minute after the iodin solution began to enter the heart. The action of iodin in the other experiments showed a tendency to cause diminished activity which was sometimes preceded by stimulation. Irregular heart action was the main effect produced in other experiments which appeared within three-fourths to two and one-fourth minutes after perfusion was begun. Depressing after effects were frequently observed (figs. 1 and 2).

EXPERIMENTS WITH 1:15,000 IODIN

The action of iodin in these experiments likewise varied. Iodin in Ringer's solution and 0.06 per cent alcohol caused in some cases a decrease in rhythm, amplitude being slightly increased at the same time. Irregular heart action, as observed with more dilute solutions of iodin, was also present, but was more pronounced. This is shown in figures 3 and 4 and in the following experiments which are typical instances of the behavior of the heart when subjected to the action of a solution of iodin 1:15,000.

Frog 145 B. IODIN 1:15,000 PERFUSED FOR THREE MINUTES

About forty-five seconds after perfusion was begun, the frequency of the heart diminished for about one-half minute, while the force of the contractions became appreciably greater, a tendency to incomplete relaxation being observed, however, at the same time as after every seventh or eighth contraction the diastole was about a quarter in extent of the one preceding or following it. Toward the end of the perfusion, every five or seven contractions were separated by a considerable interval of rest, the contractions in the interim being regular, but the amplitude steadily decreased. One and a half minutes after perfusion with iodin, the heart began to grow weaker until the contractions were hardly perceptible four minutes later. The action of iodin when used in this concentration was sometimes even more pronounced, as was shown in the following case.

A B

Fig. 1. Frog 145. *A*, heart action became irregular as a result of perfusion with iodin 1: 30,000. *B*, cardiac depression which commenced in the after period reached a maximum six minutes after perfusion with iodin was discontinued

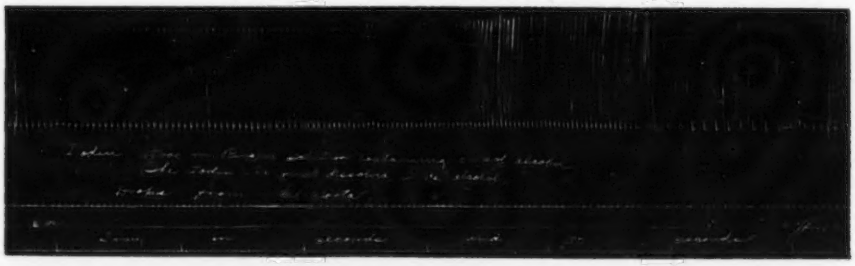


Fig 2. Frog 144. Perfusion the same as in figure 1. Initial stimulation is followed by cardiac irregularity

A B

Fig. 3. Frog 156. *A*, iodin causes decrease of frequency at first then irregular action and stimulation. *B*, four to five minutes after iodin was perfused.

A B

Fig. 4. Frog 143. *A*, marked irregularity as a result of perfusion with 1: 1,500 iodin in alcoholic-Ringer solution. *B*, two minutes after iodin. Shows depression in the after period.

Frog 143. After exposure for forty-five seconds only, cardiac activity was reduced, contractions became both feeble and irregular. Recovery was prompt, however, when iodin was discontinued. After a second perfusion for two and one-half minutes, made ten minutes later, the effect was very marked. Examination of figure 4 will show that heart action was reduced about one-third within half a minute and became irregular during the rest of the perfusion. Pronounced depression was observed after iodin was discontinued.

Experiments were also carried out in which small quantities of olive oil were added to the Ringer-iodin solution. The injury caused by iodin was much less marked in these cases. Thus, although depression

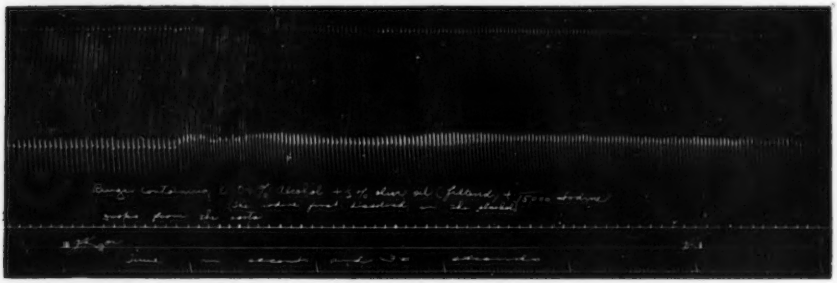


Fig. 5. Frog 149. Effect of 1: 15,000 iodin in Ringer-olive oil and alcohol. Note absence of irregularity and compare with figure 4.

was observed in one experiment in which the duration of the perfusion was six minutes and forty seconds, no effect could be noticed during the first three minutes. In another experiment in which perfusion with iodin lasted four minutes, depression appeared one and one-half minutes after the solution entered the heart. That olive oil and iodin are antagonistic was shown very strikingly in several cases in which the perfusion periods were much shorter. Thus in three tests performed on two specimens, two, three and one-fourth and three minutes were allowed for perfusion with iodin and olive oil. Little or no depression occurred during the exposure to this combination nor were after effects observed. There was on the contrary distinct stimulation in one case (fig. 5).

EXPERIMENTS WITH 1:10,000 IODIN

Within one-half to one and one-half minutes after the heart was subjected to the action of iodin and 0.08 per cent alcoholic Ringer's solution, marked disturbance of cardiac action appeared. Force and frequency varied under its influence. Systole was either moderately increased or diminished, diastole was frequently incomplete, all these changes being sometimes observed in the same experiment. In some instances, however, the action of iodin was not very marked. When perfused for two or two and one-half minutes, irregularity of action lasting about one-half minute was noticed in two cases toward the end of the experiment, a decrease of amplitude and increase of rate being observed in a third experiment after one and one-fourth minutes, while moderate increase in the force of contraction was noticed in a fourth case after the same interval of time. Depressing after effects, as already noted with more dilute solution, were more frequent when the concentration of iodin was 1:10,000. The more typical action of the heart when perfused with such a solution is shown in the following experiments:

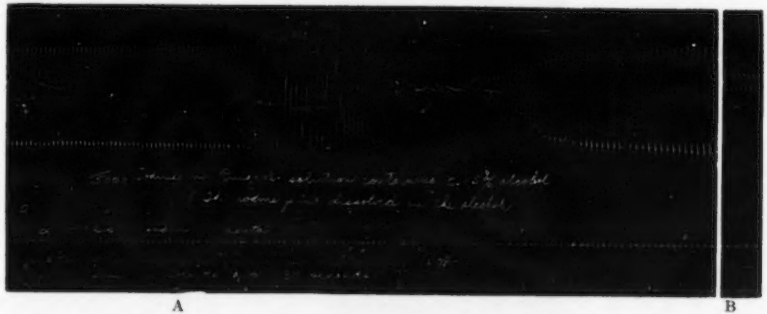
Frog 144. Perfused three minutes with 1:10,000 iodin in 0.08 per cent alcoholic Ringer's solution. Within less than half a minute heart action became irregular, periods of incomplete relaxation alternating with those of increased frequency. For two and one-half minutes after perfusion with iodin, heart action was normal, but a gradual decrease in the force of contraction set in now. Four and a half minutes later the amplitude was decreased about 70 per cent.

Frog 141. Perfused six minutes with iodin in 0.08 per cent alcoholic Ringer's solution. Within one and one-half minutes after the experiment with iodin was begun, heart became irregular, frequency fluctuated sometimes being greater, sometimes less, than before iodin. Incomplete relaxation also occurred at irregular intervals. A short period of improvement followed after iodin was discontinued, but force and frequency soon began to diminish, heart action becoming very weak in two and one-half minutes.

Frog 146. Perfused with 1:10,000 iodin in 0.08 per cent alcoholic Ringer's solution. About half a minute after the experiment with iodin was begun, irregularity of heart action preceded by a brief period of increased frequency, was observed. Within a half minute, heart action became regular once more, force and frequency being moderately increased at the same time This lasted one and one-fourth minutes

A B

Fig. 6. Frog 144. Perfusion with 1: 10,000 iodin in Ringer's solution. *A*, heart action became irregular about one minute after perfusion with iodin was begun. Note intervals of improvement. *B*, cardiac depression set in sometime after perfussion with iodin was stopped and continued steadily until it reached a maximum as shown in figure.



A B

Fig. 7. Frog 139. *A*, 1: 5,000 iodin in Ringer-alcoholic solution produced cardiac irregularity and arrest of heart in diastole; also shows recovery when iodin perfusion was discontinued. *B*, depression in after period five minutes after iodin.

when heart action became irregular again, groups of contractions separated by considerable intervals of rest being observed. The perfusion lasted four minutes. The usual depressing after effect was observed. Force of contractions began to diminish steadily after iodin perfusion.

Frog 135. Perfused two minutes with iodin in alcoholic Ringer's solution. Within one and one-half minutes after the experiment was begun the amplitude increased, but the contractions were less frequent. This was followed by a period in which the frequency and force of the contractions exhibited considerable variation. The heart was under observation three and three-fourths minutes after iodin was discontinued, but no depressing after effects were noticed. Marked effects were also observed in another test in this experiment in which perfusion

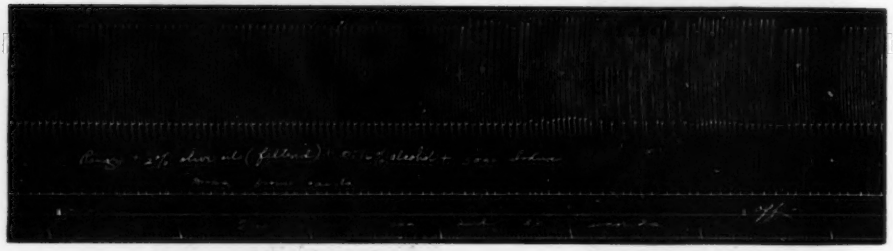


Fig. 8. Frog 153. Iodin 1: 5,000 in alcoholic-Ringer's and olive oil. Irregular heart action of short duration. Compare figure 7.

with 1: 10,000 in alcoholic Ringer's solution was carried out for one minute only. After a period of depression lasting two minutes, during which heart action was abolished for a half minute, recovery set in.

EXPERIMENTS WITH 1: 5,000 IODIN

Two series of experiments were carried out in one of which iodin was dissolved in alcoholic Ringer's solution while the other also contained olive oil. The behavior of the heart in the two cases was noticeably different. After one to two minutes perfusion with the iodin solution without olive oil, arrest of the heart in diastole was observed in two cases and a very marked decrease in the contractions occurred in the third. The other effects such as irregular heart action during perfusion and depression when iodin was discontinued were also present, and were much more pronounced than in experiments in which

weaker solutions were used. In similar tests with iodin and olive oil, the results were not quite the same. No change for two minutes could be noticed in one case, the heart suddenly became irregular, but recovery followed in less than half a minute. Marked effects were noticed in experiments on two other specimens perfused for one and one-half minutes and four and one-half minutes, but the heart never came to a standstill.

EXPERIMENTS WITH SODIUM IODID

Perfusion of the isolated frog heart with Ringer's solution to which 0.5, 1 and 2 per cent of sodium iodid were added caused depression which increased with the concentration of the iodid. This was due, however, to the increased osmotic pressure as shown in control tests with Ringer's solution to which addition of equivalent amounts of sodium chlorid produced similar effects. The action of sodium iodid was also tested by means of Ringer's solution in which the sodium chlorid was either partly or entirely replaced by equivalent amounts of sodium iodid, the composition of the solutions being: NaCl 2.1; KCL 0.075; $CaCl_2$ 0.1; $NaHCO_3$ 0.05; NaI 10.0 gms. or NaCl was entirely replaced by 15.4 gms. NaI per liter. Perfusion for one to six minutes with either of these solutions produced depression of amplitude varying in degree, rate being seldom affected (see figs. 9, 10). Recovery was almost always observed when perfusion with iodid was discontinued. In a smaller number of cases, however, sodium iodid was without any effect.

The condition of the heart seemed to be an important factor in determining the action of iodid since we often noticed marked differences in the reaction of the fresh heart and the heart that had been perfused for some time and thus approached a hypodynamic condition. As shown in figure 9, sodium iodid in Ringer's solution perfused soon after the heart was isolated was without any effect but the same treatment one hour and thirty-seven minutes later caused marked depression. The reaction of the heart to sodium iodid which was added to Locke's solution was also tested in a number of experiments. Macht stated that sodium iodid stimulated heart action, force and frequency being increased according to his records. We perfused the heart with a solution made up as follows: NaCl 4.5; KCl 0.42; $CaCl_2$ 0.24; $NaHCO_3$ 0.15; dextrose 1.0; NaI 11.5, gms. per liter, employed by Macht, or NaCl 3.0 and NaI 15.4 grams per liter, all the other ingredients being the same in both solutions. The results obtained varied con-

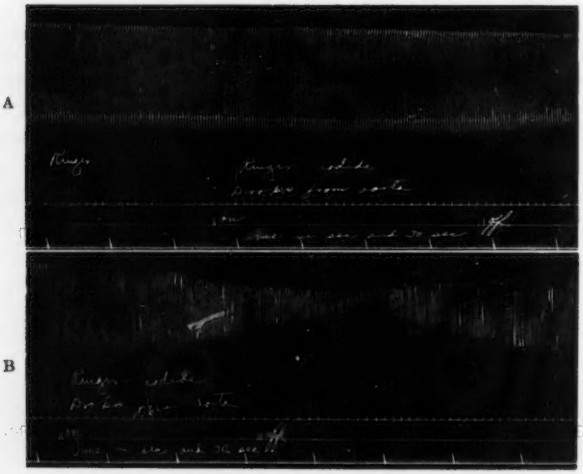


Fig. 9. Frog 189. *A*, perfusion of the fresh heart with Ringer iodid. *B*, perfusion with Ringer iodid one hour and twenty-two minutes after *A*.

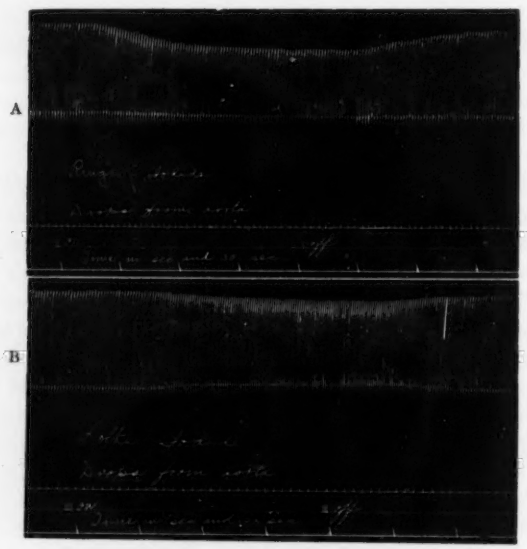


Fig. 10. Frog 197. Shows the reaction of the same heart to (*A*,) Ringer and (*B*) Locke solutions in which the same amounts of sodium iodid (15.4 grams per liter) were substituted for equivalent amounts of sodium chlorid.

siderably. Depression of amplitude was observed in some experiments (figs. 10B and 12B), in others frequency was markedly increased (fig. 12A), a moderate decrease of amplitude being present at the same time. In a third group of cases, force as well as frequency were increased (fig. 11, *A*, *B*). Occasionally the heart almost failed to react to the solution (fig. 11, *C*). The greater resistance of the fresh heart was also frequently noticed in experiments with solutions of Locke-iodide as shown in some of the figures to which reference has already been made.

DISCUSSION

The striking difference in the action of iodine and sodium iodid observed in these experiments are in harmony with the observations of previous investigators on the toxicity of these substances. The experiments of Overton (7) and those of Stockman and Charteris (8) have shown that molecular iodin is much more toxic than its sodium salt. More recently, Adler (9) studied the influence of sodium iodid and of various organic preparations on the sexual organs of the rabbit. His observations show that the alkali iodides are less injurious than organic iodin preparations.

An explanation of the action of iodin compounds had first been sought by Oswald Loeb (10). On the basis of his experiments with potassium iodid and a number of organic preparations, he formulated the lipotropic theory according to which the action of iodides depends on their solubility in the lipoids of the tissues. Support was given to it later by the work of McLean (11). He found that potassium iodid and various organic preparations when brought in contact with liver tissue *in vitro* or when administered to rabbits, are absorbed by the tissue lipoids. This would not explain, however, the reaction of the heart to iodin and sodium iodid which was observed in the present investigations, nor the enormous difference in the toxicity which has been demonstrated by several other observers. In this connection the investigations on tissue lipoids made within recent years are particularly interesting. Erlandsen (12) isolated from the heart, a substance which he called cuorin, containing acids of the linolenic and linoleic groups with an iodin number of 130.1. According to Leathes (13) the lipins of the heart contain acids which are highly unsaturated. The formation of addition products with molecular iodin may therefore be expected. The more stable product thus formed would become less readily available for cardiac metabolism since, as pointed out by Dakin (14), the

saturated fatty acids are not oxidized as readily as the unsaturated compounds. Indeed it is conceivable that the oleic acid radicle may become transformed into a more stable form with a much higher melting point and the resulting product may even become injurious. The failure to react to iodin injected into dogs intravenously may be ex-

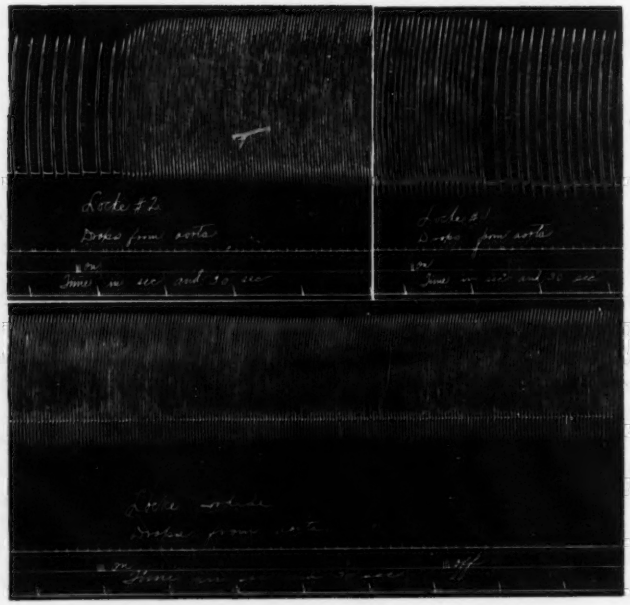


Fig. 11. Frog 185. *A*, shows stimulating effect of Locke iodid (Locke No. 2, 4.5 grams NaCl per liter replaced by equivalent amount of sodium iodid). *B*, condition of heart after seven minutes perfusion with Locke iodid and recovery when Locke No. 1 (Locke solution without iodid) was perfused. *C*, frog 198, shows only slight depression caused by Locke iodid solution (Locke solution in which 6 grams sodium chloride per liter was replaced by an equivalent amount of sodium iodid).

plained by the formation of a similar combination with the lipins of the blood, the case being analagous to the effect of iodin when olive oil was added to Ringer's solution. But this would seem to be contradicted by the results obtained in cats. It may be recalled that according to Abderhalden (15), the lipin content in the blood serum of these animals exclusive of cholesterin is about half that in the serum

of the dog's blood. Moreover, the heart being more active, probably contains acids that are more unsaturated than those in the dog heart. According to Leathes (16), the more unsaturated fatty acids are found in locations of more active metabolism. Accordingly the fat of the heart would absorb more iodin than that of the connective tissue. Hence, the more rapid the cardiac action, the more unsaturated the fats contained in the heart. Thus the iodin number of the fat obtained from the dog's heart is 70, from that of the sheep 58.

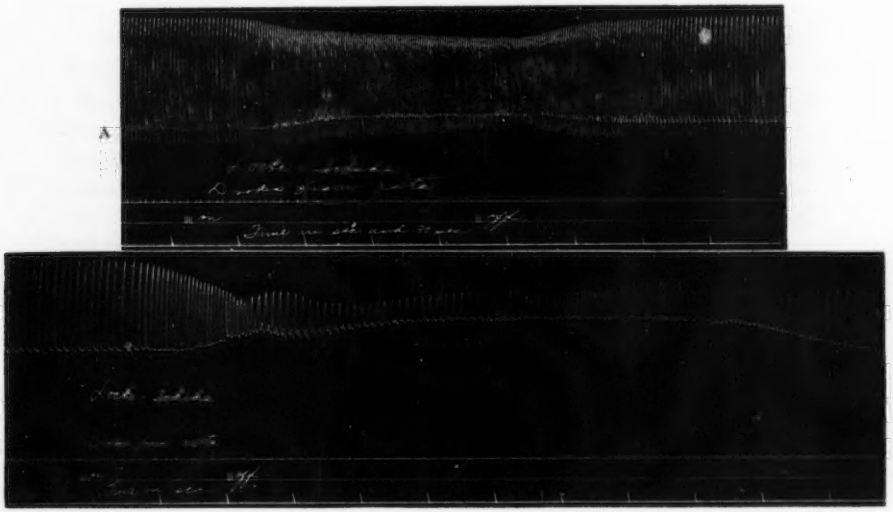


Fig. 12. Frog 191. Note reaction of heart to Locke iodid (Locke solution in which 15.4 grams NaI per liter replaced equivalent amounts of sodium chlorid.) *A*, at the beginning of the experiment. *B*, one hour and fifteen minutes after *A*.

The action of sodium iodid on the frog heart is more difficult to explain. Iodin in the ionic state does not enter into combination with the unsaturated fatty acids in a manner analagous to free iodin. There is evidence, however, that iodin is taken up from potassium iodid by the tissue lipoids. Although the experiments of Oswald Loeb (17) failed to show its presence in brain, spinal cord, and tissue fat after the administration of potassium iodid, later investigations revealed its presence in the lipoids of various organs after treatment with inorganic iodids. Thus Boruttau (18) found iodin in the brain and McLean (19)

found it in the lipoids of the liver after giving potassium iodid or after adding it to the liver *in vitro*. The mechanism in this case is probably due to physical action between the lipoids and ionic iodin.

The failure to produce any effect on the circulation after the introduction of large amounts of sodium iodid may be accounted for in part by rapid elimination, but it is also probable that it is bound in some way by the lipoids of the blood, thus preventing its action on the heart and blood vessels.

SUMMARY AND CONCLUSIONS

Dilute solutions of molecular iodin perfused through the isolated frog heart produce cardiac irregularity and depressing after effects. After more concentrated solutions, heart action becomes much more irregular and the heart may be arrested in diastole. The after effects are also more pronounced. The presence of olive oil in the perfusion fluid decreases the toxicity of iodin. Marked depression of the circulation was observed in cats after intravenous injection of iodin, but no change could be noticed in dogs similarly treated. Sodium iodid is much less toxic than iodin. A solution of $\frac{1}{2}$ per cent sodium iodid added to Ringer's solution perfused through the frog heart produced slight depression. In no case was an injurious after effect noted. Sodium iodid injected intravenously into cats and dogs failed to produce any effect on the circulation even when moderately large doses were introduced rapidly.

It is suggested that the action of molecular iodin is due to formation of additive compounds with the unsaturated fatty acids present in the frog heart. The action of iodin on the circulation of the cat might be due to the presence in the heart of large amounts of unsaturated fatty acids or perhaps to acids that are more unsaturated than those in the heart of the dog and also to the presence of smaller amounts of some of the lipoids in the blood serum of the cat than in that of the dog.

The action of sodium iodid on the frog heart is probably due to physical action with the lipoids of the heart.

Rapid elimination was suggested as a possible cause of the negative results obtained after the intravenous administration of sodium iodid to cats and dogs.

Ringer's solution in which the sodium chlorid was replaced by equivalent amounts of sodium iodid generally produced cardiac depression. The results with Locke's solution containing the same amounts of sodium iodid were not constant, sometimes stimulation and sometimes depression being observed, while in some cases no effect on the heart could be noticed as a result of the same treatment. 0.03 to 0.16 per cent alcohol in Ringer's solution had no effect on the frog heart.

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THE THROMBOPLASTIC ACTION OF CEPHALIN

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Received for publication, June 15, 1916

In 1912 Howell (1) reported the results of a study of the thromboplastic action of the tissues in which he showed that the active substance is a phosphatid having the general properties of cephalin. The effect of solutions of this phosphatid upon coagulation is very striking and it has been shown that lecithin prepared according to the customary methods is lacking in this property. The identification of the phosphatid rested mainly on its insolubility in cold alcohol and its slight solubility in hot alcohol and the possibility was recognized that the thromboplastic action might be due to some adherent impurity rather than to the phosphatid itself. At the suggestion of Dr. Howell I have undertaken a re-examination of this subject to determine if possible whether the thromboplastic effect may be attributed to an impurity, or is a property of the cephalin itself, and also to determine in how far a similar property is exhibited by other related phosphatids. The phosphatids which have been examined in regard to their thromboplastic action are cephalin, lecithin, sphingomyelin, cuorin and heparphosphatid.

THE METHOD OF TESTING THROMBOPLASTIC ACTIVITY

To determine thromboplastic activity a method was employed which has been in use in this laboratory for some time in connection with other researches on coagulation. The method rests upon the fact that in fresh serum the amount of actual or effective thrombin is small, but the amount of ineffective thrombin is relatively large. This metathrombin may be converted to active thrombin by Morawitz's (2) method of adding alkali with subsequent neutralization. But it can also be activated to effective thrombin by the addition of thromboplastic extracts, especially solutions of cephalin, provided the serum is perfectly fresh. As the serum stands the activating effect of the cephalin becomes less marked and finally disappears entirely after a certain

variable time. Long after cephalin activation has ceased entirely the alkali activation is still effective. This activating effect of cephalin on fresh serum was used in the following way. Fresh serum and fresh oxalated and centrifugalized plasma were prepared from the same animal. By trial the amount of serum was determined which would cause clotting of a definite amount of plasma within a time convenient for observation—say thirty minutes to two hours. The thromboplastic activity of cephalin or any other solution under observation was determined by adding it to the serum and noting the acceleration in the clotting time. Ordinarily the serum of dogs and cats was used and the proportions taken were two or three drops of serum to eight drops of plasma. With these proportions clotting occurs usually in from 30 minutes to 2 hours, but if to the mixture one adds a solution of cephalin or a saline extract of fresh tissue clotting occurs promptly in from 30 seconds to 2 or 3 minutes. The tests were carried out according to the following example:

Control—Oxalated plasma, 8 drops; serum, 3 drops; water, 3 drops. Clot forms in from 30 minutes to 2 hours and usually the clot is imperfect.

Solution to be tested; e.g., solution of cephalin—Oxalated plasma, 8 drops; serum, 3 drops; cephalin solution, 3 drops. A solid clot forms in one minute plus or minus.

In all of the experiments the solutions of the purified phosphatids were compared not only with a control of plasma, serum and water, but also with a control made up with an incompletely purified but very active preparation of cephalin made from the pig's brain. This form of cephalin is now used constantly in the laboratory for experimental and therapeutical purposes and for brevity's sake is designated in the following experiments as "laboratory cephalin." Its mode of preparation is as follows:

PREPARATION OF LABORATORY CEPHALIN

A fresh brain obtained from the slaughter house is freed as far as possible from membranes and blood and is then ground to a pulp in a mortar. The pulp is spread as thin as possible on a glass plate and dried in a current of warm air. When thoroughly dry the material is ground to a powder and is extracted for 4 or 5 hours with an excess of ether. The ether solution is filtered off in a closed space and is passed through the filter paper two or more times until the filtrate comes through clear. The ether filtrate is allowed to evaporate in

a current of cool air to a moist residue. The residue is extracted twice with an excess of acetone for 15 to 20 minutes and then twice with an excess of 95 per cent alcohol. Finally the residue is treated again with acetone, the acetone drained off, and the material dried in a desiccator. In using the cephalin for thromboplastic experiments a 0.1 per cent solution is made up in distilled water.

CEPHALIN PREPARED BY THE METHOD OF RENALL

Cephalin was prepared according to the modification of Parnas's (3) method proposed by Renall (4), and by which he states that a yield of very pure cephalin may be obtained. Fresh pig's brains obtained from a slaughter house are carefully freed from membranes and blood; then macerated in a mortar to a fine pulp. This pulp is placed directly nto three times its volume of acetone for a day, with occasional shaking. The acetone extract is centrifugalized off and the same amount of fresh acetone added to the residue. After remaining a day in this acetone and being subjected to occasional shaking, the insoluble portion is centrifugalized away from the acetone solution. The residue is shaken for an hour with cold absolute alcohol and the insoluble portion, after removal of the alcohol, is treated twice with three times its volume of petroleum ether of low boiling point (used instead of ethyl oxide to prevent a certain amount of oxidation which is liable to occur with the use of ordinary ether). The petroleum ether extracts, which are colorless or of a lemon yellow tinge, are concentrated in an atmosphere of carbon dioxide and precipitated by pouring into absolute alcohol. The cephalin falls as a white flocculent precipitate, which when dry has a white waxy appearance and forms with water an opalescent solution resembling that of laboratory cephalin. It possesses very active thromboplastic power as is shown in the following experiment.

Dog's oxalated plasma and dog's serum

Using—Plasma, 8 drops; cephalin, 2 drops; serum, 2 drops.
Control—Plasma, 8 drops; water, 2 drops; serum, 2 drops.

Renall's cephalin	good clot	1½ min.
Laboratory cephalin	solid clot	1 min.
Control	no clot in	30 min.

To the precipitate obtained above cerebrosides are adherent as impurities. They are removed by redissolving the precipitate in petroleum ether and allowing the solution to stand over night in the cold. The cerebrosides settle out and by centrifuging are separated from the

solution. They are washed with ether and dried. When tested in aqueous solution for thromboplastic activity they were found to be wholly negative. The petroleum ether solution is concentrated and poured into absolute alcohol—the cephalin is precipitated as a white amorphous and somewhat waxy mass, which after drying is made up into a thin emulsion with water and precipitated with normal hydrochloric acid. The acid is added to the emulsion until a good cheesy precipitate is obtained, which settles to the bottom of the container. It is collected by centrifugalization, taken up in a little ether and precipitated with acetone and dried. It forms an opalescent solution in water which upon trial shows marked thromboplastic activity as is indicated in the following typical experiments:

Dog's oxalated plasma and serum

Series I. Using—Plasma, 8 drops; cephalin, 3 drops; serum, 3 drops.
Control—Plasma, 8 drops; water, 3 drops; serum, 3 drops.

Renall's cephalin	good clot	2 min., 25 sec.
Laboratory cephalin	solid clot	2 min., 45 sec.
Control	poor clot	2 hours, 15 min.

Series II. Using—Plasma, 8 drops; cephalin, 2 drops; serum, 2 drops.
Control—Plasma, 8 drops; water, 2 drops; serum, 2 drops.

Renall's cephalin	good clot	3 min.
Laboratory cephalin	solid clot	1 min.
Control	minute clot in 5 min.	not solid in 30 min.

Thus purified cephalin prepared with a view to the prevention of oxidation and to the removal of adherent impurities possesses thromboplastic activity to a marked degree.

CEPHALIN PREPARED BY THE METHOD OF LEVENE AND WEST

Through the kindness of Dr. Levene a specimen of this cephalin was furnished in order to test its thromboplastic action. The mode of preparation of the specimen is described by the authors (5) and it will be remembered that in the quantitative hydrolysis made by them only 90 per cent of the original weight of material was obtained in the hydrolytic products. The authors therefore consider the possibility that the material contained some impurity. The sample furnished had been precipitated by hydrochloric acid from aqueous solution and after treatment with an organic solvent was finally obtained in the form of a dry powder. When this powder was dissolved in water the solution exhibited marked thromboplastic activity as is shown in the following typical experiment.

Cat's oxalated plasma and cat's serum

Using—Plasma, 8 drops; cephalin, 3 drops; serum, 3 drops.
Control—Plasma, 8 drops; water, 3 drops; serum, 3 drops.

Cephalin—Levene and West	solid clot	75 sec.
Laboratory cephalin	solid clot	40 sec.
Control	poor clot	1 hour, 30 sec.

CEPHALIN AND CUORIN PREPARED FROM THE HEART

Erlandsen (6) as a result of an extended research upon the lipoids of the heart came to the conclusion that there is no cephalin in heart muscle, but in its place he discovered a monoamino-diphosphatid related to cephalin which he called cuorin. To determine if this substance had any thromboplastic effect a quantity was prepared for trial according to the following method as given by Erlandsen:

Ox heart is freed from fat, blood vessels, peri- and endocardium and valves, ground in a meat grinder and spread upon glass plates to dry, then ground to a powder and desiccated over sulphuric acid or calcium chloride. The powdered mass is extracted with ether at room temperature, with a frequent change of the ether until all of the ether soluble material is removed. These extracts are united and concentrated to a syrup, then taken up in a little ether and allowed to stand. An insoluble fraction settles out; it is centrifugalized off and discarded. The ether solution is repeatedly precipitated with dry acetone and then with cold absolute alcohol (zero to minus 2°). This last precipitate is a dark brown sticky mass which upon dissolving in ether and precipitating with alcohol is divisible into two substances—one insoluble in alcohol at 60°, the cuorin a light brown powder, and the other appreciably soluble in alcohol at 60°, a substance having the general properties of cephalin. This cephalin was recovered from the united alcoholic extracts, which were concentrated, precipitated with acetone, taken up in a little ether and precipitated by pouring into alcohol to free from lecithin. This precipitate resembles laboratory cephalin, having the same fish-like odor, and is shown to be as active thromboplastically as the laboratory cephalin.

Cat's oxalated plasma and cat's serum

Using—Plasma, 8 drops; cephalin, 3 drops; serum, 3 drops.
Control—Plasma, 8 drops; water, 3 drops; serum, 3 drops.

Heart cephalin	solid clot	37 sec.
Laboratory cephalin	solid clot	37 sec.
Control—not clotted in 1 hour	clotted over night	

The cuorin, on the contrary when purified by repeated precipitation in alcohol at 60°, has no thromboplastic effect—indeed it possesses an anticoagulating power as may be illustrated by the following experiment.

Dog's oxalated plasma and dog's serum

Using—Plasma, 8 drops; phosphatid, 3 drops; serum, 3 drops.
Control—Plasma, 8 drops; water, 3 drops; serum, 3 drops.

Heart cephalin	solid clot 3 min.
Cuorin	not clotted in 6 hours
Control	sliding clot in 9 min.

Cuorin added to blood fresh from the artery will delay its coagulation remarkably. These two phosphatids from the heart have practically the same solubilities, they pass through repeated processes of extraction together and in the final stages are only separated from each other by the solution of the cephalin in alcohol at 60°. It is unlikely that an impurity would adhere to one of these phosphatids and not to the other.

CEPHALIN AND HEPARPHOSPHATID PREPARED FROM THE LIVER

Baskoff (7), in his work on the phosphatids of the liver, undertaken primarily for the study of the jecorin described by Drechsel (8), succeeded in isolating a phosphatid which resembles cuorin, except that analysis shows a N:P ratio of 1:1.5 instead of 1:2. To this phosphatid he gave the name of heparphosphatid. In my experiments the liver was worked up according to Baskoff's method for obtaining heparphosphatid, which is practically the same treatment to which Erlandsen subjects the heart muscle in order to secure his cuorin. The final substance insoluble in cold alcohol was further divided into a fraction insoluble in alcohol at 60° which resembles cuorin and constitutes Baskoff's heparphosphatid, and a portion soluble in alcohol at 60° which is apparently identical with cephalin. When the latter was recovered from the alcoholic solutions as in the case of the cephalin of the heart, it had all of the general properties of cephalin including the power to hasten the coagulation of blood as is shown in this experiment.

Using—Plasma, 8 drops; cephalin, 3 drops; serum, 3 drops.
Control—Plasma, 8 drops, water, 3 drops; serum, 3 drops.

Liver cephalin	solid clot 1½ min.
Laboratory cephalin	solid clot 1½ min.
Control	not clotted in 1 hour and 45 min.

The heparphosphatid on the other hand when purified by many precipitations in alcohol at 60° has no thromboplastic action and in fact shows a marked power to inhibit the coagulation. The anticoagulating action of this phosphatid is being studied and will be reported upon later. Cuorin and heparphosphatid when dry have no odor, but when moist with warm alcohol have a characteristic odor common to both. It is possible that on further purification the heparphosphatid may be shown to be identical with cuorin.

THE ACTION OF SPHINGOMYELIN

Sphingomyelin is assumed to be a diamino-monophosphatid; it is insoluble in ether and alcohol. In the process of extracting organs for phosphatids some may be carried along with the soluble phosphatids but separates out later from the etheral solutions together with their cerebrosides. The impure cerebrosides as previously mentioned were found to possess no thromboplastic action. By the kindness of Dr. Levene a specimen of sphingomyelin was obtained. When tested by the usual method it was found to have no thromboplastic activity.

THE ACTION OF LECITHIN PREPARED BY THE METHOD OF MacLEAN

Howell (1) showed clearly that lecithin as customarily prepared from the brain and from the yolk of egg exhibits no thromboplastic action. At that time there was no satisfactory method for the absolute separation of lecithin from cephalin, for cephalin though relatively insoluble in alcohol is quite soluble in an alcoholic solution of lecithin. Therefore in precipitating an ether solution of lecithin and cephalin a certain amount of the cephalin remains in solution with the lecithin. Or if the original extract of the tissue is made with alcohol much cephalin will go into solution with the lecithin. MacLean (9) has shown that "lecithin" as customarily prepared can always be separated into true lecithin, which has all of its base as choline and into cephalin with its amino ethyl alcohol base, together with other impurities. He has devised a method fully described in his article by which he has obtained lecithin with all of its base as choline. The method involves the formation of the cadmium compounds of lecithin and cephalin from the alcoholic solution of these phosphatids as obtained from the egg yolk. The separation is made on the basis of the insolubility of the lecithin cadmium chloride compound in ordinary

ether, the cephalin cadmium compound being soluble. Pure lecithin is recovered from the cadmium compound by boiling with ammonium carbonate after the manner recommended by Bergell (10). Lecithin prepared by this method when tested in the usual manner exhibited no thromboplastic activity. Attempts made to recover the cephalin from its cadmium compound were unsuccessful.

CONCLUSIONS

1. Cephalin when prepared as pure as possible exhibits marked thromboplastic activity, as indicated by its effect in increasing the thrombic action of fresh serum.

2. Cephalin exhibiting this reaction has been prepared from the liver, heart and brain.

3. The other phosphatids that have been described, lecithin, cuorin, heparphosphatid and sphingomyelin have no thromboplastic action.

4. Evidence is presented to show that this property of cephalin is not due to adherent impurities, but is a characteristic property of the cephalin itself.

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THE RAPIDITY WITH WHICH ALCOHOL AND SOME SUGARS MAY SERVE AS NUTRIMENT

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Received for publication, June 21, 1916

Alcohol has been commonly considered the most quickly available nutriment. That it is absorbed very rapidly from the alimentary tract into the blood, especially "on an empty stomach," is easily evident to one who has watched the early appearance of psychical symptoms after alcohol is taken. One reason for its early absorption is due to an appreciable proportion being absorbed in the stomach; Nemser (1) in a dog found 20 per cent of the alcohol given was so absorbed.

As the respiratory quotient offers a means of recognizing when alcohol displaces in whole or in part the other nutriments burned in the body, I have endeavored to find out with men how soon after taking alcohol (30 cc.), appreciable amounts of it are being burned. I have further extended the work to include similar experiments with the more common sugars, glucose, levulose, sucrose, lactose and maltose.

The different materials utilized by the body, if burned alone, give the following combustion or respiratory quotients: carbohydrate, 1.00; protein, 0.81; fat, 0.71; while the respiratory quotient of alcohol is 0.667. Commonly, the respiratory quotient of a normal subject, twelve hours after taking food, varies from 0.75 to 0.90 according to the varying proportions of fat and carbohydrates available and utilized in the body. If alcohol be taken, the respiratory quotient is lowered by reason of the substitution of some of the carbohydrate and probably also of the fat by alcohol in the material katabolized. Table 1 shows the average drop in the respiratory quotients in a large number of experiments made by me on men at the Nutrition Laboratory during the first two or three hours after alcohol was taken.

It may be noted in the table that the higher the initial respiratory quotient, the larger the fall after alcohol; thus either a larger amount of alcohol is katabolized to displace the other nutriments, or the alcohol

TABLE 1

Average respiratory quotients following the giving of 30 cc. of alcohol

RESPIRATORY QUOTIENT BEFORE GIVING ALCOHOL	RESPIRATORY QUOTIENT AFTER GIVING ALCOHOL	DROP DUE TO ALCOHOL
0.90	0.805	0.095
0.85	0.795	0.055
0.80	0.775	0.025

shows a preference to displace a greater proportion of carbohydrate than fat, when the initial quotient is high.

In this study, the respiratory quotients were obtained by the method of Tissot (2) the gas analyses being made on the portable Haldane apparatus (3). A mouthpiece with Tissot valves was used. The rubber tubing, joining the expiratory valve with the spirometers, was made as short as possible (air content 200 cc.), passing to a T-stopcock so that the expired air could be instantly changed from one spirometer to another. In order to find as closely as possible the time when a change in the respiratory quotient occurred, the individual periods for the first fifteen minutes following the taking of the alcohol or sugar were of three to five minutes duration.

The routine of the experimenting when alcohol was given was as follows. The subject came to the laboratory at about 8.00 a.m., having had no breakfast. He lay quietly on a couch for at least thirty minutes. Then two or three respiratory experiments were made with the Tissot apparatus, each of about ten minutes duration, to obtain the normal respiratory quotient of the morning. The subject then sat up for a few seconds and drank as a control about 200 cc. of cold cereal coffee. The subject lay down again and respiration experiments began as soon as possible; these covered periods from 1 to 4, from 4 to 7, from 7 to 10, and from 10 to 15 minutes after the coffee was taken. After ten minutes intermission, there was another period of ten minutes. Then a mixture of cereal coffee and 30 cc. absolute alcohol, the total equalling 200 cc., was drunk and similar experiments made after the alcohol as before. The control experiments were to note if taking liquid of itself affected the quotient.

The results of the experiments with alcohol are given in table 2. Eight experiments were made with five subjects. The taking of the liquid, both the alcohol and the control solution, caused an immediate rise in the quotient with all the subjects except E. It was especially marked in subject A where an average quotient of over 1.00 was ob-

tained in the first to fourth minute regardless of whether alcohol or cereal coffee had been taken. So in fixing the exact time when alcohol begins to play a prominent enough part in the metabolism to lower the respiratory quotient, it is necessary to compare the control and alcohol experiments. This rise in the quotient was probably due to a stimulation of the respiratory center of short duration (with a resulting pumping out of carbon dioxide from the body) induced by the taking of liquid.

With subject A, a distinct lowering of the respiratory quotient did not occur until the tenth to the fifteenth minute after alcohol was given; with subjects B and D a similar period was necessary; subject C showed a lowered quotient in the seventh to the tenth minute, while with subject E one finds a marked lowering as early as the fourth to the seventh minute. Thus one may observe that the time between the taking and the beginning of combustion of alcohol varies with different subjects; with some subjects the combustion may begin 4 to 7 minutes after taking alcohol, and varies with others up to 10 to 15 minutes, by which time all the subjects observed gave evidence of burning alcohol.

When sugar is fed, it replaces fat and protein in the katabolism, and in consequence one finds a rise in the respiratory quotient. Sugars thus afford one an opportunity, in the same way as alcohol, of finding how soon after taking they begin to be burned in appreciable amount in the body. In interpreting an increased respiratory quotient, when carbohydrate is given, one is forced however to admit the possibility that the rise is due wholly to the conversion of carbohydrate into fat unaccompanied by an actual combustion of the carbohydrate.

Therefore, experiments, similar in nature to those with alcohol, were made on one subject (E) who alone had shown no change in the respiratory quotient after the control solutions, to obtain data as to the rapidity with which various sugars—glucose, levulose, sucrose, maltose and lactose—began to be burned in sufficient quantities to displace other material and thus affect the respiratory quotient. The plan of experimenting was the same as with alcohol, except that control experiments were not made on the same day as the sugar experiments and that more experimental periods each day were made after sugar was given than after alcohol. One hundred grams of the sugar[1] was made up with water and cereal coffee to about 300 cc. and could be drunk

[1] An amount of sugar (about 45 grams) isodynamic to the 25 grams of alcohol would doubtless have been preferable.

TABLE 2

Respiratory quotients after a control solution and after 30 cc. alcohol

SUBJECT	BODY WEIGHT		CONTROL						ALCOHOL					
		Date	Prel.	1–4 mins.	4–7 mins.	7–10 mins.	10–15 mins.	25–35 mins.	Prel.	1–4 mins.	4–7 mins.	7–10 mins.	10–15 mins.	30–40 mins.
	kilos													
A.....	54.2	February 27, 1915.....	0.875	0.990	0.900	0.870	0.880	0.850	0.865	0.975	0.885	0.810	0.825	0.825
		March 1, 1915.....	0.890	0.965	1.050	0.965	0.900	0.890	0.890	1.030	0.950	0.955	0.895	0.790
		March 12, 1915.....	0.915	1.065	0.870	0.850	0.895	0.935	0.920	1.055	0.940	0.950	0.780	0.825
		Average.....	0.895	1.005	0.940	0.895	0.890	0.890	0.890	1.020	0.925	0.905	0.835	0.815
B.....	48.8	April 26, 1915.....	0.865	0.855	0.840	0.840	0.840	0.845	0.855	0.930	0.810	0.830	0.835	0.795
		May 7, 1915.....	0.840	0.865	0.850	0.865	0.850	0.855	0.850	0.905	0.855	0.870	0.830	0.805
		Average.....	0.850	0.860	0.845	0.855	0.845	0.850	0.855	0.920	0.830	0.850	0.830	0.800
C.....	65.2	December 3, 1914.....	0.860	0.905	0.865	0.840	0.855	0.855	0.860	0.890	0.850	0.810	0.775	0.755
D.....	66.3	April 24, 1915.....	0.825	0.845	0.795	0.800	0.825	0.825	0.825	0.865	0.800	0.810	0.800	0.800
E.....	67.0	December 5, 1914.....	0.890	0.875	0.865	0.860	0.840	0.855	0.875	0.860	0.815	0.820	0.840	0.780
		Average of all subjects	0.865	0.900	0.860	0.850	0.850	0.855	0.860	0.910	0.840	0.840	0.820	0.795

quite easily and rapidly by the subject; generally about 25 to 50 cc. of water was taken immediately afterwards to remove the excessively sweet taste from the mouth. The results of the experiments are given in table 3 and the respiratory quotients are plotted in figure 1.

The rise in the respiratory quotient after levulose and sucrose is distinctly rapid, being very marked even as early as the fourth to the eighth minute. There is a rapid rise also with lactose, but not to so marked a degree. With glucose and maltose, the definite rise in quotient does not occur until the twentieth to the thirtieth minute after the sugar was taken, although there is a small rise with maltose a little earlier.

That maltose behaves so similarly to glucose is to be expected from its being a disaccharide made up of two molecules of glucose. Sucrose on hydrolysis breaks down to give one molecule of levulose and one of glucose; probably it is its levulose content which causes the rapid rise in the respiratory quotient. Lactose, giving galactose and glucose on hydrolysis, similarly seems to owe its rapid metabolism to the galactose rather than the glucose moiety of the molecule.

This difference in action of levulose and glucose is quite in agreement with the finding of Tögel, Brezina and Durig (4); they made respiration experiments with these two sugars and found that the respiratory quotient showed a rise earlier after the levulose than the glucose. Another interesting finding in their experiments—namely the uniformly higher quotient after levulose than after dextrose—appears likewise in table 3. These workers concluded that levulose is more easily converted to fat by reason of its respiratory quotient being so often above 1.00. In this respect also, Table 3 shows that cane sugar behaves similarly to levulose and maltose to dextrose. With subject E, lactose yielded a higher respiratory quotient than dextrose, and produced a similar result with another subject at the Nutrition Laboratory; but with three others lactose led to a respiratory quotient no higher than that ordinarily found with dextrose. Lusk (5) has found little or no rise in the respiratory quotient after giving lactose to dogs.

These results with the sugars, although made upon but one subject, are substantiated in practically every detail except as above noted with lactose by a number of unpublished experiments made in another connection by Mr. L. E. Emmes and myself at the Nutrition Laboratory under the direction of Dr. F. G. Benedict, and thus make it clear that there is a fundamental and distinct difference in the metabolism of the various sugars in man and that from a nutritional point of view one

TABLE 3

Experiment with Subject E. Respiratory quotients.

DATE		AVERAGE OF PRELIMINARY	PERIOD FOLLOWING								
Dec. 5, 1914	Control	0.890	1–4 mins. 0.875	4–7 mins. 0.865	7–10 mins. 0.860	10–15 mins. 0.840			21–31 mins. 0.855			
Dec. 5, 1914	Alcohol	0.875	1–4 mins. 0.860	4–7 mins. 0.815	7–10 mins. 0.820	10–15 mins. 0.840			22–32 mins. 0.780			
Jan. 21, 1915	Maltose	0.830	1–3 mins. 0.785	3–6 mins. 0.865	6–9 mins. 0.880	9–13 mins. 0.845	13–18 mins. 0.860	18–23 mins. 0.865	23–28 mins. 0.895	35–44 mins. 0.900		
Dec. 8, 1914	Levulose	0.895	2–5 mins. 0.910	5–8 mins. 0.960	8–11 mins. 1.075	11–15 mins. 1.085	15–20 mins. 1.075	20–25 mins. 1.105	25–30 mins. 1.055	36–44 mins. 1.085		81–89 mins. 0.975
Dec. 29, 1914	Sucrose	0.885	1–4 mins. 0.835	4–7 mins. 0.950	7–10 mins. 1.030	10–14 mins. 1.085	14–19 mins. 1.125	19–24 mins. 1.145	24–29 mins. 1.135	29–34 mins. 1.110	48–56 mins. 0.995	82–90 mins. 1.025
Dec. 11, 1914	Glucose	0.870	1–4 mins. 0.815	4–7 mins. 0.910	7–10 mins. 0.855	10–15 mins. 0.865	15–20 mins. 0.865	20–25 mins. 0.895	25–30 mins. 0.910	40–49 mins. 0.940		79–87 mins. 0.965
Jan. 4, 1915	Lactose	0.845	1–4 mins. 0.915	4–7 mins. 0.875	7–10 mins. 0.880	10–14 mins. 0.900	14–19 mins. 0.940	19–24 mins. 1.015	24–29 mins. 1.015	41–51 mins. 1.010		83–92 mins. 0.950

must recognize the possible necessity of differentiating between them in their *action* and *use*. One might say in brief that levulose and sometimes galactose, judging from the respiratory quotient, shows a tendency or preference to change to fat in the body, while glucose tends to change to glycogen and be stored as such.

A study of figure 1 shows clearly that levulose, sucrose, probably lactose and possibly maltose give indications by the respiratory quotients of being metabolized, and in all probability burned by the fourth

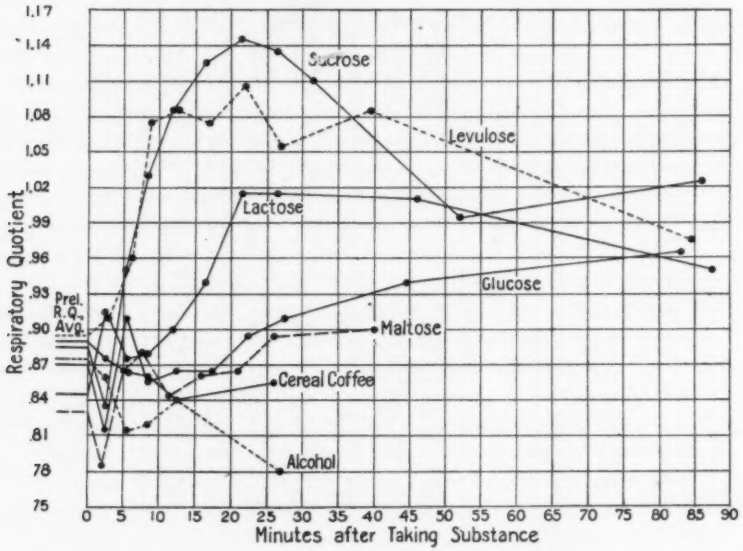

Fig. 1.

to the seventh minute after ingestion "on an empty stomach," which is quite as rapidly as alcohol in the same subject and much quicker than alcohol in four other subjects. Although alcohol is no sooner available as a food, that it will be burned in preference to sugar when the two are taken together has been shown by Tögel, Brezina and Durig (4).

SUMMARY

1. By determination of respiratory quotients in periods of 3 and 4 minutes duration upon subjects without breakfast, it was concluded that

a. Alcohol begins to be burned in appreciable quantity in from 5 to 11 minutes after taking; with some subjects the combustion began more quickly than with others;

b. Sucrose, lactose and levulose begin to be burned quite as soon as alcohol, if not sooner;

c. Glucose and maltose are not utilized as food as soon as the other sugars or alcohol, approximately twenty to thirty minutes elapsing before their combustion plays an important part in the metabolism.

2. There is a distinct difference between the metabolism in men of glucose and of levulose and galactose as shown by a study of the gaseous exchange, especially the respiratory quotients.

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